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Hemodynamic Studies of Induced Acute Hypo- and Hypervolemia in the Newborn Infant

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The following investigation was undertaken as part of a study directed toward a better understanding of the pathogenesis of the sometimes fatal complications of exchange transfusions in erythroblastotic infants. Regarding the circulatory consequences of this therapeutic procedure it was considered of interest to elucidate the mode of response of the circulatory system to changes in blood volume considerably bigger than those normally induced during replacement transfusions in order to obtain more detailed information about th adaptive capacity of the cardiovascular apparatus of the newborn. As the blood pressure response to changes in blood volume is related to the compliance of the vascular bed as well as to its ability to redistribute the available blood volume information regarding the vasomotor characteristics of the newborn infant might be gained in this manner

Although our concept of circulatory regulation in the newborn infant is largely based on studies in animals as well as on inference from our knowledge of circulatory regulation in adult man some basic observations have also been made directly in the newborn infant. The capacity of the newborn to secrete adrenalin and noradrenalin stimulated by change from the lying to the upright position has been verified [18]. Plasma adrenalin and noradrenalin levels have also been observed to be elevated during respiratory difficulties in the newborn [9]. The finding of normal adult levels of catecholamines in various fetal organs [17] has further corroborated that there is ample production of adrenergic material.

The effect of various stimuli, including catecholamines, on the circulation of the newborn child has been investigated both with respect to pulmonary [1 22] and systemic blood pressure [1]. From these studies it seems clear that the end-organ in the peripheral vascular bed is capable of the same qualitative response as in adult man. Quantitative evaluations of the regulation of peripheral resistance have been made [8 29] and with the pletysmographic technique t has been possible to quantitate peripheral perfusion in the extremities as well as to demonstrate an impressive capacity to control peripheral vascular resistance [7].

The blood volume of the newborn infant

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may vary a great deal depending upon the amount of blood transfused from the placenta before the cord is clamped [16 40], and there has been speculation as to whether a disturbed circulatory homeostasis due to interference with this physiological transfusion may play some role in the pathogenesis of the respiratory distress syndrome of the newborn infant [28].

Apart from the desire to get more detailed information regarding the functional capacity of the circulatory system of the newborn it was thus also hoped that studies of the hemodynamic consequences of hypo- and hypervolemia might shed some light on the importance of the physiological placental transfusion to the neonate in the maintenance of circulatory homeostasis in the period immediately following birth

Material and Methods

Fifteen erythroblastotic infants varying in age from 11 hours to 6 days and in birthweight from 2390 to 4800 g were studied immediately prior to an exchange transfusion. None of the infants was, at the time of investigation, in circulatory failure as judged by clinical appraisal, roentgenologic examination of the heart and lungs and the level of central venous pressure Hemoglobin values were in the range 16–19 g% indicating that none of the investigated patients was excessively anemic or hemodiluted. Blood transfused was of the Rh neg type in the case of Rh immunization and in the case of ABO immunization was usually of O type blood corpuscles in homologous (baby type) plasma. The average hematocrit of administered blood was 55 %. Nine infants were studied with respect to changes in blood pressure heart size and heart rate during variations in blood volume. In the remaining six cases, radiologic determination of the heart size alone was done in two cases and in the remaining four cases heart rate and blood pressure were studied. Right atrial pressure was studied in four cases, right ventricular pressure in four cases, aortic pressure in four cases, PCA pressure left atrial pressure and pulmonary artery pressure in one case each. In three patients simultaneous pressure recordings were made from more than one location. The infants were as a rule breathing quietly and not aroused by the procedure

All studies were done with the exchange catheter introduced through the umbilical vein and lodged with its tip 3–6 cm up the vein. The pressure recording catheter or catheters, were introduced either through the umbilical vessels to the various cardiovascular sections studied or if these could not be reached this way through the saphenous vein. Aortic pressures were recorded with the catheter introduced through the umbilical artery to the abdominal aorta. The recording catheters were of the Cournand type and pressures were recorded over a strain-gauge manometer with the signal fed over an amplifier to a photographically recording instrument Zero reference was kept at level of the anterior axillary line Heart rate was calculated from simultaneous ECG tracings on the recording paper The radiographic determination of the heart size was based upon films exposed in the frontal and lateral views of the thorax and was calculated with the aid of the ellipsoid formula of Rohrer [30] and Kahlstorf [22] as product of the three diameters and a constant The diameters of the roentgenogram of the heart in frontal projection were calculated from the film that showed the larger cardiac dimension of a pair of two exposures. To obtain satisfactory depth measurement of the heart a small amount of barium contrast was given orally to outline the esophagus. The constant used was 0 40 and includes the ellipsoid formula constant and a factor to compensate for the distorsion resulting from the divergency of the rays. The exposure time did not exceed 0.006 seconds, and the outline of the heart was not blurred. No estimate of the random error of the method was made but earlier investigations per

formed under similar conditions [24] indi cate that it does not exceed 12 % f the mean.

The verage irculating blood volume f the patients was considered t be about 110 ml/kg body weight, figure suggested by a series of blood volume determinations made by the uthors in another group of eryth roblastotic infants (range 86–1 6 ml/kg) This figure is in good greement with reports in the literature [3].

After initial observations f the various parameters under study blood volume changes were induced by the step-wise withdrawal of 60 to 120 ml of blood or to the equivalent of approximately 25 % of the estimated blood volume of the patient in question. Pressure recordings were made at intervals, in five cases after each 10–20 ml removed, in the remaining cases after removal of 40–60 ml and when withdrawal had been completed. In some instances a new measurement was made when 10–15 minutes had elapsed after the removal of blood, t determine the possible influence of time upon the induced pressure changes. Heart-size determinations were usually made when half f the planned total withdrawal had been performed as well as when blood loss was at maximum. When the planned total withdrawal was completed the infant was transfused t restore the initial blood volume and new measurements were then mad

A hypervolemic stat was then induced by the step-wise addition of an amount of blood equal to that which had previously been removed. Recordings were made at intervals as in the hypovolemic state. After a series of final recordings the exchange transfusion was completed in the usual manner

Results

During the reported experiments the infants behaved normally the only change in appearence noted was that the skin during hypovolemia appeared pale and during hypervolemia red purple. The various recorded values are listed in Table 1 For typographical reasons the table has been limited to the data at every *0 ml removed or added. Although few in num ber the experiments allow of some conclusions, as the recorded consequences of the induced volume changes were uniform in all cases studied.

Heart size

The radiographically determined heart size changed markedly with the amount of circulating blood. At an average reduction in blood volume of 25 % the corresponding average reduction in heart volume was 30 % (range 1.–42 %) of the initial observation. Expressed in ml the heart-size reduction was on an average 14 ml (range 8– 1 ml) With restitution of blood volume the heart size returned to the initial value Hypervolemia on the other hand caused an increase in the heart volume of 18 % of the initial observation (range 3–46 %) corresponding to an average increase of 6.5 ml (range –18 ml) in the nine cases studied. Intermediate observa tions indicate that the change in heart size is roughly proportional to the amount of blood added or withdrawn.

The variations in heart size were paralleled by similar alterations in the degree of vascularity of the lungs as judged by a subjective estimate of the width of th central lung vessels without actually measuring them.

Heart rate

The heart rate increased from an aver age of 130/min at the beginning of the experiment to an average of 171/min during maximal hypovolemia. In the five cases where intermediate recordings were made after every 10–20 ml removed, an

TABLE 1 *Heart volume, frequency and blood pressure response to induced volume changes*

All pressures are expressed in mm Hg. Heart volume in ml
RA right atrium; LA – left atrium; RV – right ventricle; AO – aorta; PA – pulmonary artery;
PCA – pulmonary capillary arterial pressure.

Case	Birth weight g	Age	Observed paramet	Init. obs.	−20 ml	−40 ml	−60 ml	−80 ml	−100 ml	−120 ml	Restit	+20 ml	+40 ml	+60 ml	+80 ml	+100 ml	+120 ml
1	2950	3 d	H vol	43				26									
2	3290	29 h	H vol	64			50									48	
3	3600	24 h	H vol	63					42				63			65	
			Freq	120					180							135	
			RA	+1.6					−1.4							+7.5	
4	4800	2 d	H vol	67						46							72
			Freq.	150						180							150
			RA	+6.9						−2.1							+10.2
5	3270	12 h	H vol	44					26		42					51	
			Freq	125			164		176		140					135	
			RA	+1			−1		−2		+6					+17	
			LA	+1			0		−2		+7					+7	
6	3010	5 d	H vol	41			35		32		41					48	
			Freq	110			150		160		130					120	
			RA	+2.7			+0.8		−[illegible]5		+9					+17	
7	4100	4 h	H vol	67				59			69						
			Freq	120				185			140						
8	4200	6 d	H vol	39		32			[illegible]		41		83			87	
			Freq	110		125			170		150		90				
			[illegible]	/													
	[illegible]		vol/Freq.	[illegible]		[illegible]			[illegible]		[illegible]		[illegible]			[illegible]	
											[illegible]		[illegible]		[illegible]		

			RV	26/2		28/0.6			23/−5	37/3		56/10			03/10
				(11)		(9)			(7)	(15)		(29)			(20)
9	2290	2 d	H vol	22				19		35				39	
			Freq.	105	140	145		170		65		100		90	
			AO	66/53	76/47	73/47		41/26		87/64		110/78		115/85	
				(61)	(55)	(53)		(33)		(73)		(95)		(96)	
10	3730	2 d	H vol	30					22	36					40
			Freq.	120					140	140					120
11	2606	3 d	H-vol	34		29		28		34				42	
			Freq	170		178		180		180				145	
			PCA	+14		+12		+8		+14				+26	
			AO	61/58		46/28		40/20		84/50				100/71	
				(61)		(31)		(26)		(70)				(75)	
12	2540	11 h	Freq.	122	125	122	125	140		123	130	120	113	111	
			RV	60/7	56/6	46/2	34/1	26/1		60/7	67/13	71/18	77/23	76/23	
				(32)	(29)	(16)	(8)	(9)		(32)	(37)	(44)	(46)	(47)	
13	3550	24 h	Freq	135	146	156	176	180		160	146	143	146	143	
			RV	69/2	61/0	53/ 1	56/ 1	85/ 1.5		74/11	79/16	77/17	76/15	86/18	
				(37)	(30)	(28)	(27)	(20)		(42)	(49)	(46)	(50)	(52)	
			AO	67/56	56/45	49/37	38/28	34/24		78/60	75/62	78/60	73/57	76/59	
				(51)	(46)	(41)	(34)	(27)		(65)	(59)	(64)	(60)	(64)	
14	2000	36 h	Freq.	158	171	176	171	180		136	133	130	127	117	
			PA	33/9	37/18	32/13	24/9	13/6		34/17	36/13	42/26	62/34	66/43	
				(20)	(25)	(23)	(14)	(7)		(25)	(27)	(40)	(46)	(50)	
15	2650	48 h	Freq	165	180	195	190								
			RV	36/3	33/0	18/ 3	16/−5								
				(20)	(17)	(10)	(7)								

acceleration was noted as the first 10 ml were withdrawn in four while in the remaining case no appreciable increase in heart rate was observed until all of the planned volume had been removed. During restitution of blood volume the heart rate decelerated and in most cases approached the initial values. Hypervolemia caused little change in heart rate with only a minor further reduction in rate in a few cases.

Right heart filling pressure (RHFP)

This term denotes right atrial mean pressure or right ventricular end-diastolic pressure In the eight cases in which one of these values or both were recorded there was a fall in pressure from a mean of +3.2 mm Hg to a mean of −2.4 mm Hg associated with the withdrawal of the stated volume of blood, whereas induction of hypervolemia to the same relative magnitude increased the right heart filling pressure to a mean value of +14 mm Hg Values recorded during the restitution of blood volume between these two states usually were somewhat higher than the initial observations.

Right ventricular pulse pressure

In the four cases in which this pressure was observed during blood withdrawal there was a drop from an average of 48 mm Hg to 31 mm Hg and in the three cases where hypervolemia was subsequently produced, the pulse pressure rose to an average value of 58 mm Hg

Pulmonary artery mean pressure

If the PCA mean pressure accepted as indicative of the level of the pulmonary artery mean pressure It was possible to obtain a value for the latter in two cases In these the influence of changes in circulating blood volume was clearly demonstrable The initial pulmonary artery mean pressures of 14 and 20 mm Hg dropped to 8 and 7 mm Hg respectively during hypovolemia and mean pressures of 26 and 50 mm Hg respectively were recorded during maximal hypervolemia.

Pulmonary artery peak pressure

As in the absence of cardiac malformation right ventricular systolic pressure is identical to pulmonary artery peak pressure this value was recorded in five of the cases. There was a pressure fall from an average of 47 mm Hg to an average of 30 during maximal hypovolemia and an increase to 72 mm Hg during the maximal hypervolemic state

Aortic pressure

Systolic as well as diastolic pressures in the abdominal aorta fell to less than half of the initially recorded values at maximal hypovolemia in the three cases studied while the consequences of hypervolemia were somewhat less pronounced, although the aortic pressures rose to well above the initial levels.

Discussion

The influence of changes in blood volume upon circulation has been studied to some extent both in man and in animals Before the introduction of the heart catheterization technique Wallace & Sharpey Schafer [30] studied the effect on the systemic blood pressure and heart rate of the withdrawal of 600–1000 ml of blood

from healthy volunteers. If the subjects lost more than 1000 ml, or amounts roughly corresponding to 20–25 % of the estimated total blood volume (TBV) the systemic blood pressure was only slightly affected in eight cases, with an average drop of 11 and 1 mm Hg in systolic and diastolic pressures respectively In the remaining five cases a marked drop in both systolic and diastolic pressures was observed. No systematic pulse reactions were observed during the experiment. Another group of authors [31] report that the systemic pressure decreased by an average of slightly over 20 mm Hg in systole during the withdrawal of 1000 ml of blood from supine volunteers. This group also reported that the venous pressures as well as the pulse rates were relatively uninfluenced by the procedure

As pressure is the product of flow and resistance, changes in blood pressure during hypo- or hypervolemia must indicate that the induced volume changes have exceeded the adaptive capacities of the peripheral vasomotor system and the cardiac output. The adaptability of the vascular bed to changes in TBV has been studied experimentally by Bazett [3] who reports that moderate degrees of hypovolemia, corresponding to a blood loss of 20–30 % of the TBV in the dog were associated with an increase in the peripheral resistance sufficient to maintain systemic pressures, while cardiac output remained constant. Similar experiments [25] suggest that the compensatory increase in peripheral resistance is capable of maintaining the systemic blood pressure in man if the blood loss does not exceed 700–1000 ml. The increase in peripheral resistance results chiefly in a redistribution of blood away from the skin, kidneys and the splanchnic region [25]. Wiggers *et al* [41] studying the effect of bleeding in the dog suggest that the capacity to increase peripheral resistance is limited to states of moderate blood loss and that when heavy blood losses are induced this mechanism no longer functions, with life-endangering circulatory consequences.

Cardiac output together with peripheral resistance the regulator of blood pressure is intimately connected with the vasomotor control of the peripheral vessels which governs the venous return to the heart. Ever since the law of Starling was formulated [33] much discussion has centered upon the regulation of cardiac output and there is as yet no complete agreement regarding the role played by the right heart filling pressure in the regulation of cardiac stroke volume Even if this pressure is not the only regulating factor most studies of hypovolemia have demonstrated that a venous hypotension is associated with decrease of the stroke volume [10 15 27].

Although most investigations of the hemodynamic consequences of changes in TBV have concerned hypovolemic states, some reports pertaining to hypervolemia are also available Such studies have indicated that when TBV is rapidly increased by 20–25 % of the estimated initial volume venous hypertension occurs [23 35 39] unaccompanied by significant changes in systemic pressure and pulse rate [35 39]. In adult man an increase in pulmonary artery mean pressure occurs and is related to the degree of hypervolemia [35]. Although the RHFP has been reported to increase during hypervolemic

experiments in man, no significant changes have been observed in the right heart stroke volume [35 39]. Hypervolemic studies in the dog have shown a somewhat different mode of response with proportional increase in left ventricular end-diastolic pressure and left ventricular volume and also a moderate increase in systemic arterial pressure [20 21] during moderate degrees of hypervolemia.

From the cited literature it seems clear that the vasomotor activity in healthy man in supine position undergoing blood volume changes of ±20% of TBV is capable of adapting total vascular volume to total available blood volume so adequately that in most instances the systemic pressures are only moderately affected and cardiac output is maintained whereas the pulmonary circuit is more sensitive to these changes.

Although visual observations of the infants studied in the present investigation revealed that the vascular compartments of the skin participated in the redistribution of blood during the volume changes hypovolemia draining blood from the cutaneous vessels and hypervolemia causing pooling of blood in the same vascular compartments, the marked effect on systemic blood pressure in contrast to the cited findings in adult man suggests a less adaptive circulatory system a more rigid cardiovascular basin The relative inability of the cardiovascular apparatus to cope with hyper and hypovolemic conditions reported here is also reflected in the marked changes in the RHFP which together with the impressive changes in heart size and pulse pressure in the systemic as well as in the pulmonary circulation indicates an effect on the stroke volume Induced changes in the degree of vascularity of the lungs are also interpreted as supporting this view The pronounced pulse acceleration encountered during hypovolemia, again in contrast to the findings in adult man, may be interpreted as an effort to maintain an adequate cardiac output in the presence of a decreased stroke volume

If the systemic pressure consequences of alterations in TBV are more pronounced in the newborn infant than in adult man, the effect upon the pulmonary circulation seems much the same with hypotension following hypovolemia and hypertension following hypervolemia.

Judging from the clinical impression of the infants studied, the induced pressure changes did not affect the patients adversely during the investigation At the moment of birth, however when the infant is participating in a hemodynamic experiment that requires a great deal of adaptability of the circulatory system a possible adverse effect of induced hemodynamic changes may be accentuated. The central hemodynamic event at birth is the opening up of new vascular beds in the lungs at the initiation of breathing whereupon the pulmonary vascular resistance suddenly falls [12] and blood is shunted from the aorta into the pulmonary circulation over the ductus arteriosus It may also be assumed that the sudden drop in environmental temperature and pressure during delivery induce changes in the peripheral resistance. At this moment when the cardiovascular apparatus in the newborn should be working hard to maintain circulatory homeostasis and the left heart is increasing it stroke volume in a struggle to maintain adequate systemic perfusion in the pre-

sence of a left–right shunt in ductus arteriosus [11], an induced hypovolemia or hypervolemia may quite conceivably jeopardize the circulatory adaptation to extrauterine life. The fact that in most instances this adaptation proceeds not only successfully but also with an amazingly well stabilized systemic pressure level [19 37] demonstrates that the adaptive processes during birth are adequate providing the necessary venous return to the heart. Judging from the data in the present investigation, this would mean that the actual blood volume excess or deficit arising during adaptation to extrauterine life would be less than 20–40 ml, a figure that is probably already exceeded by the increment in pulmonary blood volume at the start of respiration. The probable explanation of this discrepancy is, that situations of hyper or hypovolemia during and immediately after delivery are counteracted not only by "active" cardiovascular adapt tion but also by the availability of a means of mobilizing blood from and/or into a blood reservoir should the need present itself. Such a reservoir may be pools of blood within the infant s body or it may consist of the fetal part of the placental circulation. The former alternative is not well studied but the latter has been investigated to some extent. In support of the circulatory significance of the placental transfusion, the effect of late versus early clamping of the cord has been shown to have a pronounced effect upon the pressure levels in the neonate [34] as well as upon the blood volume [13 40] and the size of the heart [6]

As early as in 1876 Budin [5] suggested that the clamping of the umbilical cord ought to be delayed in order to create the best possible conditions for the institution of normal pulmonary function in the neonate This has been re-emphasized by others [4 28 37] later and further corroborated by the fact that a higher incidence of respiratory troubles was reported in infants whose umbilical cords were ligated immediately after delivery [26] The finding of a significantly smaller TBV in infants who develop respiratory distress [14] may also be cited in support of the importance of the placental transfusion for the institution of normal pulmonary function.

As the clinical dysfunction as well as the pathological changes in early respiratory distress are localized to the lungs the possible influence of an imbalanced circulation upon pulmonary dynamics should be discussed. Hypervolemia due to over transfusion from the placenta is not an immediate threat during delivery as it requires active stripping of the umbilical cords therefore the following discussion will be limited to instances of hypovolemia.

Although the pulmonary artery observations are few it may be assumed that the observed changes in systemic circulation reflect alterations in the flow and pressure in the pulmonary circulation as well as these two circulatory systems are intimately connected to each other in the immediate neonatal period. The question may be raised as to whether the hypotensive reaction in the pulmonary artery during hypovolemia also interferes structurally with the aeration of the lung parenchyma. If the vascular bed of the lung is considered as a reinforcing mesh work for the parenchyma it is easy to appreciate the importance of the pulmonary pressure

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TABLE 1 *Weight and some laboratory findings in serum of patient M.K. at different ages*

Age (yr) ...	2	[illegible]/12	4	[illegible]/12	[illegible]/12	7 [illegible]/12
Weight, kg	10.0	12.1	1[illegible]1	8.6		
Alkaline phosphatase (Buch & Buch), units	19	26	34 66	22	30	33
Bilirubin, mg/100 ml					0.5	0.[illegible]
Meulengracht index	1 8	1 8	1 8			
Thymol turbidity test, O.D. at 720 mμ	0.06	0.07	0.06		0.20	
Transaminase GOT units					[illegible]9	20
Protein, g/100 ml			6.5	7.4	6.8	6.3
Sodium, mEq/l					148	14[illegible]
Potassium, mEq/l			3.1	—	1.6	[illegible]0
Calcium, mEq/l	4.8	4.9	7.0 4.7	7.0	5.7	5.8
Chloride mEq/l	120		95	114	106	116
Total bicarbonate mEq/l			[illegible]1	4	15	14
Phosphate-phosphorus, mg/100 ml	3.6	[illegible]7	2.5 4.1	0.7	0.9	0.6

preference for salty food. Table 1 shows how her body weight progressed. According to the mother the child's abdomen continued to grow larger and the vulvar region started to swell a few months after birth, whilst her limbs remained skinny. At the age of two years she was first admitted to the Children Hospital, Gothenburg, where she was subsequently examined annually from 1951 to 1954. When first seen it was apparent that among other things, the child had severe rickets, which had set in notwithstanding routine vitamin D prophylaxis. When she was three a cyst-like defect of unclear etiology was discovered in the left femur. Two years later she sustained a fracture through the defect and was forced to remain in bed. The skeleton underwent progressive decalcification and new multiple fractures and severe thoracic and other deformities ensued.

Whenever examined, the child was found to have not only grave rickets but also hepatosplenomegaly and dystrophy. The liver was nodose and palpable a fingerbreadth or two below the costal margin; the spleen was two or three fingerbreadths below it. Laboratory findings are listed in Table 1. Thrombocytopenia (58,000–136,000) and mild leucopenia were present. At the age of two the sternal marrow exhibited slightly reduced erythropoiesis. Liver function tests were consistently normal. The renal function was somewhat impaired; concentration tests done on two occasions before age four yielded urinary specific gravities of 1.016 and 1.020. The urine contained no albumin or merely traces, glucosuria was never detected.

The girl was last admitted to hospital (Uddevalla Lasarett) at the age of 7 after having been ailing for more than a year but being remarkably resistant to infectious diseases. Laboratory findings are given in Table 1. She now had normal or raised hemoglobin and erythrocyte values, mild acidosis, high or normal calcium, extremely low phosphorus and moderately elevated alkaline phosphatase levels in serum. No cystine crystals could be detected in the cornea. Chromatography disclosed massive aminoaciduria. At 8 years old she weighed less than 9 kg. She suffered greatly from respiratory distress and died of pneumonia in September 1957.

Post mortem examination

The body (length 83 cm, weight 8.4 kg) was emaciated and showed pronounced skeletal deformities characteristic of rickets. The long bones were bowed and short with enlarged epiphyses. There was pronounced inward projection of the costochondral junctions and the spine was kyphotic and scoliotic. The abdomen appeared distended. There was no jaundice (Fig. 1).



Fig. 1. Case M. K. at autopsy. Note skeletal deformities (bowed upper arms and ribs, kyphoscoliosis), large nodulous liver, enlarged kidneys and spleen.

Heart. Weight 95 g. Large compared to weight with slight dilatation of ventricles, especially the right. Valves, myocardium, coronary arteries, aorta and pulmonary artery were normal.

Lungs. Purulent tracheobronchitis. Bilateral atelectasis, broncho-pneumonia. Pleural cavities normal.

Liver. Weight 255 g. Only slightly enlarged but extremely distorted, being composed of yellowish brown nodules, 1–5 cm in diameter and separated by alternate narrow and broad septa. Gall bladder thin walled, bile ducts normal (Fig. 1).

Pancreas. Normal.

Spleen. Weight, 63 g. Enlarged with slightly thickened capsule. Pulp dark red and firm. Malphigian corpuscles barely distinguishable.

Kidneys. Weights: right 195 g, left 135 g. Grossly enlarged especially in proportion to body weight. Capsules stripped easily, surface smooth with delicate vascular network. The cortex was pale, 6–8 mm broad, clearly demarcated from the medulla and with thin bluish red streaks radiating towards the periphery. The pyramids were congested. The pelvis, ureters and bladder were normal.

Genitals. Ovaries, total weight 1.1 g, normal. Uterus, tubes, vagina and external genitals, normal.

Alimentary tract. Normal.

Endocrine glands. Pituitary, weight 230 mg, normal. Thyroid, weight 3.0 g (after fixation in 10% formalin) normal cut surface. Parathyroids: only two glands found, possibly enlarged. Adrenals: weight 5.0 g, apparently enlarged relative to body weight.

Brain. Normal.

Lymphoid system. Thymus, slightly atrophic (weight 2.3 g). Some soft, about peanut sized lymph nodes in the mediastinum and mesentery.

Microscopic examination

Liver. The parenchyma was composed of rounded nodules, circumscribed and separ



Fig. 2. Part of liver nodule. The multilobular pattern is clearly visible. Hematoxylin-eosin, ×30

ated by coarse and narrow strands of fibrous tissue. Each nodule was made up of several liver lobules with slightly irregular spacing of the portal canals and efferent veins. The septa contained small groups of bile ducts and scattered inflammatory cells, mainly round cells but occasional polymorphonuclear leucocytes. The picture corresponds well with that of coarse nodular cirrhosis (post-necrotic scarring of the liver, toxic cirrhosis of Mallory). Most of the liver cells contained small lipid droplets and, in addition, fairly pronounced fatty changes surrounded some central veins. No signs of malignancy (Fig. 2, 3).

Spleen. Slight broadening of the sinus walls as a sign of passive congestion.

Kidneys. The glomeruli appeared normal. The proximal convoluted tubules were greatly enlarged and had large epithelial cells showing granular cytoplasm and hyaline droplet degeneration. A few tubules showed flattened epithelium and contained hyaline cylinders with a negative amyloid reaction when stained with methyl violet (Fig. 4).

Adrenals. The enlarged organs had a markedly convoluted cortex with distinctly outlined glomerular and fasciculär zones. The marrow was well preserved.

Parathyroids. Composed of solid masses of clear cells, they contained sparse fat tissue. The largest cut surface of the two glands measured 3 × 5 mm and × 6 mm.

Thymus. Atrophic.

Lymph nodes. Slight to moderate signs of chronic, nonspecific inflammation.

Distal femoral epiphysis. The marrow was composed only of fat tissue. The epiphyseal cartilage plate was widened, thickened, markedly uneven and irregularly penetrated by branching blood vessels from the metaphyseal end of the shaft, a picture characteristic of rickets.

Other organs. Brain: Acute congestion. Pituitary, thyroid, dura mater, choroid plexus, myocardium, aorta, pancreas, ovaries and uterus normal. Esophagus, stomach, duodenum, jejunum, ileum and colon: essentially normal (post-mortal changes).

No cystine crystals were found in any organ when alcohol-fixed sections were examined in polarized light.

Case A.K. The patient was born at term on September 4th, 1956, weighing 2700 g



Fig. 3. Part of another liver nodule showing vacuolization after dissolved fat droplets, and groups of bile ducts and scattered round cells in the adjacent fibrous tissue. Hematoxylin-eosin. × 190.

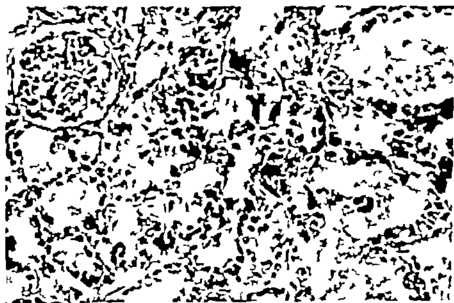


Fig. 4. Kidney with greatly enlarged tubules with altered epithelial cells. Hematoxylin-eosin. × 190.

TABLE *Weight and some laboratory findings in serum of patient A.K at different ages*

Age (yr)	5/12–7/12	8/12	10/12	1	16/12	2	3	3
Weight kg	6.2 6.4			8.6	10.0	11.3	13.2	16.5
Alkaline phosphatase (Bodansky & Bech), units	41 44	35	23	25	23	21	9	10
Bilirubin, mg/100 ml	1.2	0 1	1 1	0.4	0...			0.6
Thymol turbidity test, units	1.8		1 7					
O D at 720 mμ						0.06	0 0*	0.01
Transaminases GOT unit	160	47		68	42		30	10
GPT unit							15	18
Protein, g/100 ml	4.6 4.4 6.1	6.6	6.8		6.7	6.9	7 6	7.0
Sodium, mEq/l	150	143	146		142	143	145	144
Potassium, mEq/l	4.5	4.7	5 0	4.6	4.9	4.7	3.7	3.3
Calcium, mEq/l	5.6 5.2 6.3	5.2	6.6	6.8	5.8	6.2	5.9	5.1
Chloride mEq/l	120 122	111	112		110	114	105	107
Total bicarbonate, mEq/l	15 20 18	18	16	17	18	16	17	22
Phosphate-phosphorus, mg/100 ml	3 2 2.6 2.8	4.9	4.5	4.3	3.7	3.5	3.0	1.9

and appeared normally developed. Pregnancy and parturition were uncomplicated. Her mental development was never abnormal. She was breast-fed exclusively for six weeks and then received supplements of cow milk. Vitamin D prophylaxis was commenced after two weeks with a daily dose of about 1000 I.U. In due course the girl started to exhibit distension of the abdomen, swelling of the vulvar region and thin limbs—that is, precisely the same manifestations as her eldest sister. Consequently she was admitted to hospital (Uddevalla Lasarett) at the age of 5 months. Her weight was then normal (6190 g), but during the 2½ months before discharge she gained only 230 g, probably to some extent owing to emotional factors.

Physical examination disclosed hepato-splenomegaly (nodose liver palpable two and spleen palpable one or two fingerbreadths below the costal margin) and craniotabes. X-ray evidence and laboratory findings (Table 2) furnished further conclusive indications of rickets. Other laboratory examinations revealed moderate anemia (7 4–10 g % Hb) with slightly reduced erythropoiesis and mild relative neutropenia and lymphocytosis. Platelets, 182,000. Liver function tests (Table 2) showed normal thymol and bilirubin concentration in serum but elevated GOT values and in addition excessive alkaline phosphatase levels, though the latter probably reflected the patient's rickets. Furthermore the protein and total bicarbonate concentrations of blood were reduced, whilst other blood electrolytes were normal. Glucosuria, proteinuria and polyuria were absent but the urinary excretion of amino acids was raised (see below); the urinary phosphate excretion was not determined.

The patient was treated with vitamin D in large doses, altogether six million I.U being given from March 21 to April 11 1957 whereupon she was given 30 000 I U every sixth week. This treatment seemed to heal the rickets, and no signs of rickets have been seen during an observation period of five years. The deciduous teeth were small but apparently normal. Notably however the serum phosphorus level was abnormally low and tended to fall.

The patient's development in other respect has been reasonably satisfactory. She learnt to walk at 12 months. Her build may be on the slender side, but her weight has consistently been rather low though not subnormal. Gradually the abdomen acquired a normal configuration, the vulvar edema subsided

and the hepatosplenomegaly became less palpable. However the vulvar edema recurred sporadically. At 5 years old both liver and spleen were palpable a fingerbreadth below the costal margin. Liver function tests carried out regularly since the patient was a year old were consistently normal. The hematological picture showed persistence of slight relative neutropenia and lymphocytosis but no anemia. The platelet count was 0,000. Glucosuria albuminuria and polyuria were still absent but aminoaciduria persisted (see below).

Amino Acid Metabolism

Method

The methods described by Jagenburg [15] were used for determination of amino nitrogen, *total nitrogen, creatinine and amino acids*. The ion exchange method of Spackman, Stein & Moore [23] was used for quantitative estimation of individual free amino acids. The 150 cm column was usually run at 50 but for determination of free amino acids of tissues the pH 3.25 buffer was run at 30 C. No quantitative study of the basic amino acid was performed. Protein was precipitated with picric acid [25] and tissue extracts were prepared as described by Tallan *et al* [29].

The total amount of tyrosine and its derivatives was determined by the Millon reaction, using tyrosine as standard.

p-Hydroxyphenylpyruvic acid was determined as the borate complex according to Hsia's procedure [14]. This method also determines other hydroxy acids such as phenylpyruvic acid the presence of the latter being ruled out by a negative ferric chloride test.

The occurrence of the tyrosine derivatives was established by paper chromatography of *an ether extract obtained by continuous* extraction of acidified urine. The following solvent systems were used (*A*) iso-propanol (200 ml) conc ammonia (10 ml) water (20 ml) and (*B*) benzene (125 ml) acetic acid (72 ml) water (3 ml). Both one- and two-dimensional chromatograms were made; (*A*) was used as first solvent (*B*) as second. The spots were located with diazotized sulphanilic acid, diazotized *p*-nitraniline and silver nitrate reagent [**]. Commercial *p*-hydroxyphenylpyruvic acid and *p*-hydroxyphenyl acetic acid were used as references. *p*-hydroxyphenyllactic acid was synthetized from L-tyrosine according to Kotak [17].

Results

Case M.A. The *urinary amino acid* excretion 6 months and 1 month before death was extremely high amino nitrogen amounting to 1 and *1 of total nitrogen. There was an almost uniform increase in the excretion of the individual amino acid. However the *urinary amino acid pattern* was more compatible with the plasma pattern than with that of normal urines. The excretion of proline was negligible. The tyrosine excretion per g total nitrogen was 70 and 03 mg on the two occasions.

The *plasma amino acid* were normal or low (Fig 7). The calculated clearances indicate that the tubular reabsorption of most of them was very incomplete there *being hardly any reabsorption of glycine* and alanine. Proline was the only amino acid whose reabsorption seemed normal i.e. almost complete.

The excretion of *tyrosine metabolites* was not determined.

The amounts of *free amino acids in the liver spleen and heart muscle* were similar to those in a control dying of a non metabolic disease (Table 3).

Case A.K. The *urinary amino acid excretion* was followed from the age of 5 months to the age of 5 years and 8 months. Altogether 13 urine samples were analyzed most of them night urines. The individual amino acids in six samples were determined

TABLE 3 *Free amino acids and related compounds in different tissues from patient M.K. and from a patient dead of a non metabolic disease*

μ moles per kg wet tissue

Compound	Liver: Patient M.K.	Liver: Control	Heart: Patient M.K.	Heart: Control	Spleen: Patient M.K.	Spleen: Control
Taurine	0.63	0.58	3.01	2.49	7.13	5.41
Hydroxyproline[a]	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Aspartic acid	0.20	0.15	0.49	0.16	4.04[b]	[illegible][b]
Threonine	0.74	0.47	0.28	0.54		
Serine + glutamine + asparagine[c]	0.98	1.66	7.07	5.13	3.06	2.3
Proline[a]	0.5	0.5	0.2	< 0.2	2.0	1.0
Glutamic acid + glutathione[d]	3.44	4.18	2.43	[illegible]	6.09	6.5
Glycine	2.83	2.80	1.52	.73	5.06	5.27
Alanine	6.60	6.12	3.54	4.85	2.80	[illegible]
Amino-butyric acid	0.31	0.13	< 0.15	< 0.15	< 0.15	< 0.15
Valine	0.95	0.60	0.23	0.22	1.63	1.16
Cystine	< 0.15	< 0.15	< 0.15	< 0.15	0.45	0.45
Methionine	0.20	0.09	< 0.15	< 0.15	0.44	0.33
Isoleucine	0.43	0.41	0.22	0.14	0.87	0.60
Leucine	0.96	0.79	0.30	0.27	[illegible]	1.17
Tyrosine	0.32	0.33	0.17	0.26	0.76	0.48
Phenylalanine	0.37	0.39	0.15	0.24	0.84	0.49
β Alanine	0.69	0.47	< 0.15	< 0.15	< 0.15	< 0.15
β-Amino-iso-butyric acid	< 0.15	< 0.15	< 0.15	< 0.15	< 0.15	< 0.15
Ornithine[a]	1.5	1.5	< 0.3	< 0.3	1.5	1.5
Lysine[a]	2.0	2.0	< 0.3	< 0.3	2.5	2.0
Histidine[a]	0.3	0.3	< 0.3	< 0.3	0.3	0.3
1 Methylhistidine[a]	< 0.3	< 0.3	< 0.3	< 0.3	0.3	0.3
3-Methylhistidine	< 0.3	< 0.3	< 0.3	< 0.3	0.3	0.3
Arginine[a]	0.3	< 0.3	0.3	0.3	< 0.3	< 0.3
Ethanolamine[a]	2.0	2.0	0.7	0.7	[illegible]	[illegible]

[a] Approximate values determination by two-dimensional chromatography

[b] Paper chromatography showed about equal aspartic acid and threonine amounts.

[c] Calculated as serine. Glutamine partly destroyed during preparation of sample and chromatography

[d] Calculated as glutamic acid.

quantitatively (indicated by arrows in Fig 5).

The amino nitrogen excretion was consistently excessive but varied considerably whether related to the excretion of total nitrogen or to that of creatinine (Fig 5)

Among the individual amino acids there was, with few exceptions, a uniform increase in the excretion (Fig 6) Thus the tyrosine excretion consistently was more enhanced than the excretion of the other amino acids. The excretion of tyrosine per g total nitrogen varied from 4 to 128 mg (Table 4) parallelling the changes in amino nitrogen excretion (cf. Fig 5).

The methionine excretion was remarkably high in samples collected at 5 and 6 months of age when 28 and 15 mg methionine were excreted per g total nitrogen. Subsequently the excretion was below 7 mg

Before the age of four only traces of valine and isoleucine were detected, but

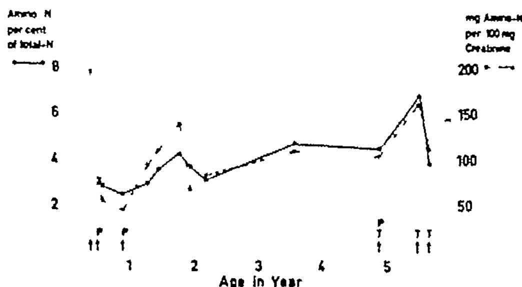


Fig. 5. Urinary amino nitrogen excretion in patient A.K. in relation to total nitrogen and creatinine excretion at different ages. Arrows indicate that the individual amino acids were determined quantitatively in the corresponding urine sample. T indicates tyrosine metabolite determination of urine. P indicates free amino acid determinations of plasma.

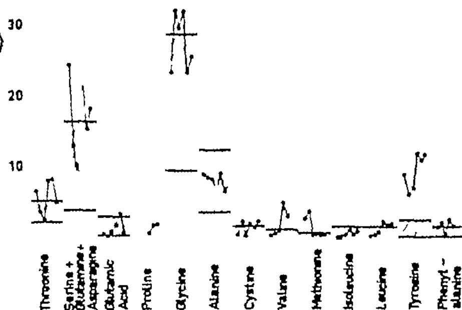


Fig. 6. Urinary amino acid pattern on six occasions in patient A.K.; amino acid nitrogen expressed in per cent of total amino nitrogen. Comparison with normal pattern (shaded area). The figure gives changes in the pattern with increasing age (from left to right).

TABLE 4 *Urinary excretion of tyrosine and tyrosine metabolites (in mg) in relation to total nitrogen excretion (in g) in patient A K at different ages*

Age (yr) —	$\frac{1}{12}$	$\frac{6}{12}$	$\frac{11}{12}$	$4\frac{11}{12}$	5_{12}	5_{12}
Millon positive substances	—	—	—	94	149	107
Tyrosine	103	*8	4	75	1*8	5
p-Hydroxyphenylpyruvic acid	—	—	—	16	19	26
p-Hydroxyphenyllactic acid + *p*-Hydroxyphenylacetic acid*	—	—	—	3		6

Determined as the difference between Millon positive substances and tyrosine plus *p*-hydroxyphenylpyruvic acid.

their excretion subsequently increased. These changes in the amino acid pattern were combined with appearance of small amounts of proline No proline was found in prior samples.

Plasma amino acid Blood for plasma amino acid determinations was drawn on three occasions viz., at 6 months 11 months and 5 years of age (Fig 7) The tyrosine level was markedly elevated, mean 4.8 mg per 100 ml, i.e almost five times normal [5 35] Apart from the very high level of methionine and the moderately increased level of the compounds contributing to the threonine–serine peak at 6 months of age the concentrations of the other amino acids were normal or not significantly elevated

Tyrosine metabolites The mean excretion of Millon positive material—calculated as tyrosine—was 117 mg per g of total nitrogen (Table 4) on average 79 being tyrosine 18% *p*-hydroxyphenylpyruvic acid and 3% undetermined. Paper chromatography established that ether extracts of the urine contained considerable amounts of *p*-hydroxyphenylpyruvic acid plus small and similar amount of *p*-hydroxyphenylacetic acid and *p*-hydroxyphenyllactic acid roughly in accordance with the quantitative figures.

No dihydroxyphenylalanine or tyramine was demonstrable paper chromatographically

The parents and unaffected sister had normal plasma amino acid patterns and normal urinary amino acid excretion.

Discussion

Most likely the two sisters suffered from the same perhaps hereditary disease The non-consanguinous parents and a sister were apparently not affected and showed no abnormalities in amino acid metabolism In three of the four similar cases referred to in the introduction, the parents were relatives [*0 27 28] and in one case a brother died, aged * months, of liver cirrhosis [*0]

Post mortem examination showed that the liver damage in patient M.K. was of the coarse nodular cirrhosis type also called post-necrotic scarring The cases of Baber [1] and Royer *et al* [17] apparently had liver changes of the same type The size of the nodules in the case reported by Sakai *et al.* [30] suggests that this patient too had similar liver cirrhosis. In the fourth case [*0] the cirrhosis was described as periportal, as judged from biopsy material In all these cases the present included,

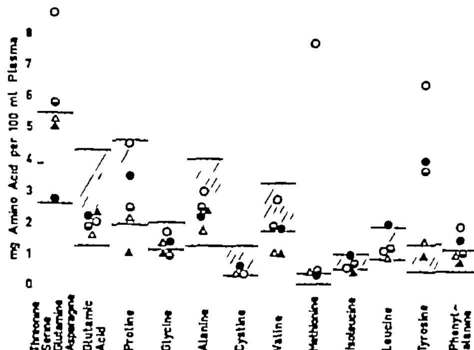


Fig. Plasma amino acid levels in patient M.K. and A.K. compared with normal levels (shaded area). Patient M.K. 7 years, 8 months; patient M.K., age 8 years, 1 month; ○ patient A.K., age 6 month; patient A.K. age 11 months; ● patient A. K., age 4 years, 11 months.

...ere was fatty infiltration of the parenchyma.

In two cases [20 30] the cirrhosis at the age of about five years terminated in malignant hepatoma a not too uncommon sequel to cirrhosis of this type [cf 13].

When a in the present cases there is no history of poisoning or infection of the liver according to Himsworth [10] one should consider nutritional factors. He postulated that the chronic loss of amino acids especially cystine in hereditary diseases associated with renal aminoaciduria might give rise to nutritional liver damage. However liver damage was not observed in several recently described syndromes characterized by generalized renal aminoaciduria [22 23 26] In the condition known to be associated with the greatest loss of cystine—cystinuria [7 38]—liver cirrhosis is not seen. Furthermore the loss of sulphur-containing amino acids owing to tubular malabsorption was comparatively small in the present cases. It therefore seems unlikely that the liver cirrhosis developed on a nutritional basis because of the renal tubular defects.

The high blood level and high excretion of tyrosine in addition to the high excretion of *p*-hydroxyphenylpyruvic acid, indicate that the tyrosine metabolism might be disturbed. The single metabolic block which could explain such an excretory pattern is in step * (Fig 8). A balanced diet can be estimated to contain about 500 mg of tyrosine plus phenylalanine per g protein nitrogen [8]. If the block is complete about the same amount of tyro-

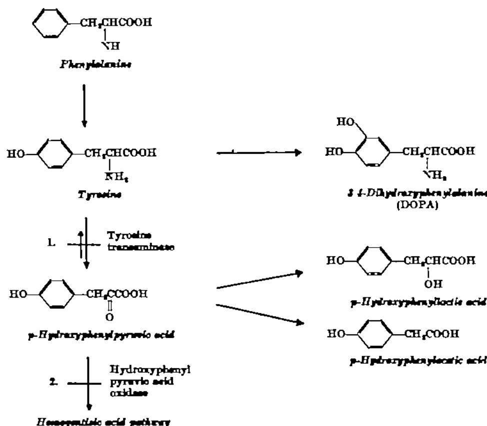


Fig. 8. Schedule of tyrosine met bolism.

sine plus tyrosine metabolites must be excreted per g total nitrogen, as experi mentally verified [11] However in patient M.K. on an average only 116 mg of Millon positive material was excreted per g total nitrogen, indicating that the block was incomplete

In subjects with an assumed decrease in the activity of the p-hydroxyphenyl pyruvic acid oxidase it has been found that the excretion of p-hydroxyphenylpyruvic acid is about equal to [11 28] or even higher than the excretion of tyrosine [1 -5] In patient A.K. the p-hydroxyphenyl pyruvic acid excretion was only one fourth that of tyrosine This could be explained by assuming that there was also a defective tyrosine transaminase step (Fig 8)

A block in step 2 is also said markedly to increase the excretion of p-hydroxyphenyl lactic acid and p-hydroxyphenylacetic acid [11 28 cf. 34 pp 389–390], although Medes [25] did not demonstrate increased amounts of these compounds in her case. In the present case the excretion of these compounds were only slightly enhanced

A disturbed tyrosine metabolism could be a consequence of any liver damage In various liver diseases it has been observed that the excretion of p-hydroxyphenyl-pyruvic acid sometimes is moderately increased [9 12] The tyrosine level of the

blood [4], as well as its urinary excretion [10], can also be somewhat increased but is usually not obviously changed in different types and stages of liver cirrhosis in adults [6, 40].

In diseases such as hepatolenticular degeneration [7, 37] and cystinosis [7, 8], sometimes associated with liver cirrhosis, no defects in tyrosine metabolism have been demonstrated. Nor were any signs of impaired tyrosine metabolism noted in a male adult with liver cirrhosis and tubular defects [38].

In gross liver damage with necrosis the amino acid level of the blood is elevated, which is reflected in the urine by incremental excretion [40]. Among observed changes in plasma amino acids an elevated methionine level seems the most consistent sign of acute hepatic necrosis [10, 31].

At 5 months old patient A.K. showed a very high urinary amino acid excretion with a remarkably high proportion of methionine. The high output of methionine, which persisted at the age of 6 months, was shown to be due to an elevated plasma level. Because the concentration of methionine in blood and the subsequent urinary excretion were normalized, one must assume that a necrotizing liver process was going on at an age of 5–6 months although the liver function tests were but slightly abnormal. When the child was first seen a nodular liver was palpable, indicating that the liver must have suffered from necrotizing processes even before the age of 5 months.

As the high excretion of tyrosine and *p*-hydroxyphenylpyruvic acid persisted in spite of the disappearance of the other signs of hepatic failure, it cannot be excluded that the disturbed tyrosine metabolism was primarily due to a genetic defect which secondarily gave rise to the liver damage.

In spite of certain deviations, patients M.K. and A.K. presumably suffered from the same disease as the clinical features were similar except that A.K. was less seriously affected than M.K. This difference may partly be due to the fact that A.K., unlike M.K., was treated from an early age with large doses of vitamin D. Both patients had about the same excretion of tyrosine in relation to total nitrogen but M.K. showed no increase in the plasma tyrosine level. As is evident from the low urinary nitrogen output of about 2 g per day in this patient, the protein intake must have been very low, which is in agreement with the clinical observation that she had refused most food for a considerable time. The intake of tyrosine and phenylalanine must therefore have been very inadequate. It is well known that the plasma phenylalanine level in phenylpyruvic oligophrenia can be normalized by reducing the intake of this amino acid [34]. The normal plasma tyrosine level in patient M.K. therefore does not seem to contradict the opinion that she had a disturbed tyrosine metabolism, especially as the loss of this amino acid in the urine was great due to the tubular defects.

Post mortem there were but low amounts of free tyrosine in the liver, spleen and myocardium. The patient of Sakai *et al.* who showed similar tyrosine excretion to the present patients but very much higher *p*-hydroxyphenyllactic acid excretion, reportedly had very great amounts of tyrosine in the liver [30]. No such increase was found in two other patients with an apparently complete lack of *p*-hydroxyphenylpyruvic acid oxidase activity [11].

The kidneys are described only in two [1 30] of the four similar cases reported. In Baber's [1] case the kidneys were grossly enlarged but had remarkably small alterations microscopically. Sakai *et al* [30] found enlarged kidneys microscopically showing tubular nephrosis with cells of granular structure a picture very similar to that in our case

As in cystinosis it is hard to explain the functional tubular defects and the lesions in the kidney if one assumes that the primary abnormality is a defect in an enzyme system mainly located to the liver The assumption that some of the accumulated metabolites may inhibit renal enzymes of importance for the tubular reabsorption has not been verified. Recently Krebs [18] demonstrated that *p*-hydroxyphenylpyruvic acid may act as a potent inhibitor of renal glucogenesis. However this observation cannot directly explain the decreased tubular reabsorption.

Summary

Two siblings with a syndrome clinically characterized by liver cirrhosis, renal defects, and vitamin D resistent rickets are described. The non-consanguinous parents and a sister were not affected. Both patients had generalized aminoaciduria of mainly renal origin. The younger sister had a remarkably high excretion of tyrosine a marked elevation of the plasma level of this amino acid, and an excessive excretion of *p*-hydroxyphenylpyruvic acid indicating a partial enzymatic block at the *p*-hydroxyphenylpyruvic acid oxidase step The elder sister who died at 8 years of age was studied only late in the disease She exhibited no increase in the plasma tyrosine level, presumably owing to inadequate protein intake Post mortem examination revealed wasting and pronounced skeletal deformities characteristic of rickets coarse nodular liver cirrhosis with moderately pronounced fatty change enlarged congested spleen, grossly enlarged kidneys with tubular nephrosis; proportionately large adrenals with cortical hyperplasia and possibly enlarged parathyroids composed of solid masses of clear cells. The possibility that the primary metabolic disturbance might be a defect in the tyrosine metabolism i discussed.

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Studies on Erythro-Kinetics in Infancy

IV The Long Term Behaviour of Radioiron in Circulating Foetal and Adult Haemoglobin and its Faecal Excretion

by LARS GARBY STIG SJÖLIN and JEAN-CLAUDE VUILLE

Previous studies on the relative rate of synthesis of haemoglobin F and A in infants by Garby Sjölin & Vuille [7] have shown that intravenously injected radioiron is incorporated into both haemoglobin A and haemoglobin F during the first months of life Five of the infants studied were followed up for periods ranging from 58 to 123 days after injection of the isotope The age at injection was between 5 and 25 days. In all five cases the total amount of radioactivity present in the circulating total haemoglobin (haemoglobin A and F) was recorded at time intervals of about one week. In three of the infants the excretion of radioiron in the faeces was also measured. The present paper comprises a description of the behaviour of radioactive iron in such long term studies. The results have bearing on several aspects of ferro-erythro-kinetics in infancy and extend and corroborate earlier findings by us [8] concerning the short-term behaviour of radioiron in this age group. Furthermore a new approach is presented for calculating the daily excretion of iron in young infants.

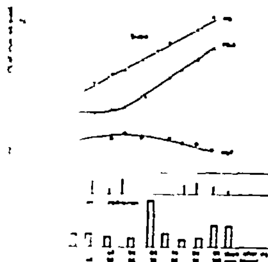
Material

The clinical data concerning the five patients La, Bl, Sj Lö and Ja have been presented in a previous paper [7]. All were considered to be haematologically normal (infant Lö had rather low haemoglobin concentration during the latter part of the study and will be discussed in greater detail). Two of the infants, Bl and J suffered from myelomeningocele and hydrocephalus, but they had no further complications during the period of investigation. All infants were fed with breast milk during the first 2 to 4 weeks of life; they were subsequently given different cow-milk mixtures providing a daily intake of iron of 1 mg/kg body weight

Methods

The methods used to measure the amount of radioactivity present in total haemoglobin, haemoglobin A and F have been reported [6, 7 8]. In calculating the blood volume correction factor was introduced in order to compensate for the frequent blood sampling. This factor amounted to 5–10% of the total blood volume at the end

This investigation was supported by grants from the Swedish Medical Research Council and by grants (Research Project 154/RB) from the International Atomic Energy Agency Vienna.



Haemoglobin during the first months of life after ... The reticulocyte count and the faecal excretion ... also shown.

... activity in ... more rapid ... injection ... up ... increase in ... for more iron. The ... increase in ... first but ... this time ... precursor iron ... the first ... Thus the increase of ... in circulating haemoglobin

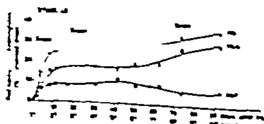


Fig. 4. Case LC. Total iron in circulating haemoglobin during the first months of life after intravenous injection of Fe[59] at the age of 1 d.

during the second to third month of age is compatible with the assumption of a considerable increase in haemoglobin production. The magnitude of this increase and the absolute rate of haemoglobin synthesis cannot be evaluated on the basis of the present data (cf. Garby, Sjölin & Vuille [ ]). As can be seen from the figures there is good correlation between the onset of the third slope of the curve and the rise in reticulocyte values. A transient reticulocytosis, both relative and absolute, at about two months of age has also been observed by Gairdner, Marks & Roscoe [4] and by West [1]. In the former study, a concomitant increase in marrow erythroid count was found. This count, however, continued to increase although the reticulocyte count again fell to values below 1 ... at about three months of age.

Case LC, in whom the appearance of the third slope was delayed, deserves special comment. This infant had suffered from postnatal hyperbilirubinaemia (maximum value 22.3 mg/100 ml on the sixth day of

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M terial

The clinical data concerning the five patients Li, Bl, Sj Lö, and Ja have been presented in a previous paper [7]. All were considered t be haematologically normal (infant Lö had a rather low haemoglobin concentration during the latter part of the study and will be discussed in greater detail). Tw f the infants, Bl and Ja, suffered from myelomeningocele and hydrocephalus, but they had no further complications during the period f investigation. All infant were fed with breast milk during the first 2 to 4 weeks of life: they were subsequently given different cow milk mixtures providing a daily intake of iron f 1 mg/kg body weight.

Methods

The methods used to measure the mount of radioactivity present in total haemoglobin, haemoglobin A and F have been reported [6, 7 8]. In calculating the blood volume, correction factor was introduced in order t compensat for the frequent blood sampling This factor amounted t 5–10% f the total blood volume at the end

of the investigation period. The amount of radioactivity given were as follows: Case Li 0.3 μC, Case Bl 0.6 μC, Case Sj 0.5 μC, Case Lö 1.6 μC and Case Ja 8.0 μC. The radiation dose received from the isotope has been given in a previous paper [ ].

Measurement of the radioactivity in faeces. Each week all stool-containing diapers were collected during three consecutive days. The whole mass of diapers and faeces was then wet-ashed with concentrated sulphuric acid and nitric acid. The procedure was continued until all organic material had disappeared. The salt-rich suspension was further concentrated by boiling to a final volume of about 20 to 30 ml and then quantitatively transferred into several (4–6) plastic tubes fitted to the well type scintillation detector. The error of the radioactivity counting was rather large because of the relatively small amount of the isotope that had been injected, the coefficient of variation being about 5 in Cases Bl and Ja and between 5 and 20 in Case Sj. The amount of radioactivity excreted per day was expressed as per cent of the total activity injected.

Reticulocyte counts were performed by Seip [15] method but siliconised glassware was not used.

Results

Radioactivity in haemoglobin. The amount of radioactivity present in circulating total, foetal and adult haemoglobin, the reticulocyte counts and the excretion of radioiron are presented in Fig. 1–5 as function of the time after injection of the isotope. The age of the infants is also indicated.

In four of the infants (Li, Bl, Sj and Lö) the curves of the activity in total haemoglobin showed three more or less distinct slopes. After an initial uptake period lasting for some 11–17 days (slope 1) during which 25–35 of the injected dose was incorporated into circulating cells there was a much lower increase for 15 to 44 days (slope 2). When the infants were between 40 and 70 days of age a more rapid incorporation of the isotope could again be observed. The final values for red-cell radioactivity were 43 in Case Li at the age of f days, 80 in Case Bl at the age of 130 days, 73 in Case Sj at the age of 100 days and 37 in Case Lö at the age of 110 days.

The fifth case, Ja, differed in some respects from the others. The initial uptake period (slope 1) lasted for about 15 days but during this time more radioactivity, about 55, was incorporated. This initial uptake period was followed by a steady increase in radioactivity, however, and no second slope was discernible. The final radioactivity at the age of 120 days was 87 .

During the initial uptake period (slope 1) the radioactivity was almost equally divided between HbA and HbF in Case Li, Bl and Ja, whereas in Case Sj and Lö a considerably greater part was found in HbA. After this period the activity in HbF did not in any case increase significantly and 50–75 days after the injection a slight decrease had occurred in all cases except Li, where no decrease was discernible at the end of the investigation 60 days after the injection (Fig. 1). After the initial uptake period the activity in HbA increased more or less parallel to the increase in total haemoglobin. In Cases Li, Bl, Sj and Lö a definitely faster rate of increase was noted 35 to 55 days after injection (Fig. 1–4). In Case Ja there was a steady increase in HbA activity after the initial uptake period.

Reticulocytes. In Cases Bl, Sj and Ja in which reticulocyte counts were made frequently a definite increase in the reticu

locyte count was noted during the second month of life

Radioactivity in faeces The faecal excretion of radioiron showed a similar pattern in all three infants studied. During the first 15–20 days after injection the daily excretion was 0 1 % to 0.25 % of the injected dose. Between days 20 and 60 a relatively constant level of 0 005 % to 0 04 % was observed. In Cases Bl and Ja peaks of 0 1 % and 0 06 % respectively were noted at about the 100th day after injection. Infant Sj showed two different peaks, the higher (0 17 %) at about 60 days and the other at about 90 days after injection

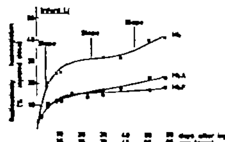


Fig. 1 *Case Li.* Radioiron in circulating haemoglobin during the first months of life after intravenous injection of Fe^{59} at the age of 5 days.

Discussion

Radioactivity in circulating haemoglobin. The curves of the radioactivity in circulating total haemoglobin give further support to the previous conclusions [8] that the synthesis of haemoglobin is considerably lower during the first one to two months of life than during the first two to four days of life and that during the second or third month of life there is a gradual increase in bone marrow activity Initially not more than 25 to 55 % of the injected radioiron appeared in the circulation (corresponding value during the first days of life 80–100 %) but when the infants reached the age of 1½ to ½ months,

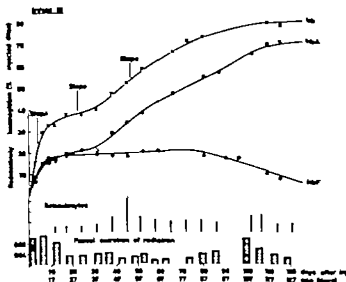


Fig. 2. *Case Bl.* Radioiron in circulating haemoglobin during the first months of life after intravenous injection of Fe^{59} at the age of 7 days. The reticulocyte count and the faecal excretion of radioiron are also shown.

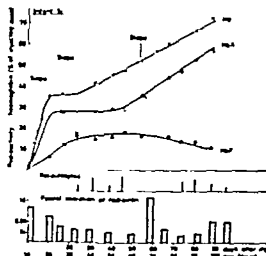


FIG. 3. *Case SJ.* Radioiron in circulating haemoglobin during the first months of lif after intra enous injection of F 59 t the age of 16 days. The reticulocyt count and the faecal excretion of radioiron are also shown.

the rate of appearance of radioactivity in the peripheral blood became more rapid again. During the first days after injection a considerable proportion of the radioactive atom are thu taken up by iron stores, to be released when the increase in bone marrow activity call for more iron. The third slope of the curve corresponding to the increase in bone-marrow activity is certainly much less steep than the first but it must be borne in mind that by this time the specific activity of the precursor iron is very much lower than during the first days after injection. Thu the increase of *radioactivity in circulating haemoglobin* during the second to third month of age is compatible with the assumption of a considerable increase in haemoglobin production. The magnitude of this increase and the absolute rate of haemoglobin synthesis cannot be evaluated on the basis of the present data (cf Garby Sjölin & Vuille [8]). As can be seen from the figures there is good correlation between the onset of the third slope of the curve and the rise in reticulocyte values. A transient reticulocytosis both relative and absolute at about two month of age has also been observed by Gairdner Marks & Roscoe [4] and by West [17]. In the former study a concomitant increase in marrow erythroid count was found. This count, however continued to increase although the reticulocyte count again fell to value below 1 at about three month of age.

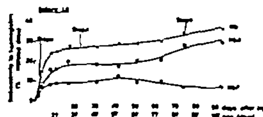


Fig. 4. *Case LÖ.* Radioiron in circulating haemoglobin during the first months of lif after intravenous injection of Fe59 t the age of 17 days.

Case LÖ, in whom the appearance of the third slope was delayed deserves special comment. This infant had suffered from postnatal hyperbilirubinaemia (maximum value 22.3 mg/100 ml on the sixth day of

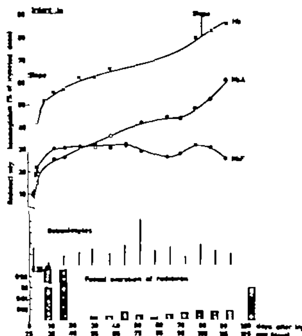


Fig. 5. Case Ja. Radioiron in circulating haemoglobin during the first months of life after intravenous injection of Fe[59] at the age of 25 days. The reticulocyte count and the faecal excretion of radioiron are also shown.

life): there were no signs of haemolytic disease however and no exchange transfusion was performed. During the whole course of the study the infant appeared perfectly healthy though he had a rather low haemoglobin concentration (around 9 g/100 ml) throughout the second and third months of life. In view of the behaviour of the labelled iron, this anaemia can be explained by an unusually long delay in the onset of bone-marrow recovery. We do not know whether the neonatal hyperbilirubinaemia may have had anything to do with this phenomenon but it is perhaps worth mentioning that West [17] claimed to have found lower reticulocyte values during the first three weeks of life in full term infants with hyperbilirubinaemia (more than 7 mg/100 ml) than in infants whose bilirubin concentration was less than 7 mg/100 ml. It is also of interest to note that Labbe Zaske & Aldrich [13] found evidence of inhibition of haem synthesis by bilirubin *in vitro*

Infant Ja showed an exceptionally high uptake of radioiron in the peripheral blood, and had elevated reticulocyte values throughout the investigation period. In the study on the relative rate of synthesis of haemoglobin F [7], this infant seemed to synthesize much more HbF than normal full-term infants, and also had a consistently higher proportion of circulating HbF (in this respect behaving like a premature infant) These two extreme cases Lö and Ja must probably be regarded as reflecting individual variations in the time-course of the changes in erythropoiesis

In all infants the curves for radioiron in Hb A and HbF showed a divergent pattern after the initial uptake period. The radioactivity in Hb F remained on a more or less constant level, whereas the activity in Hb A increased continuously. This is in accordance with the findings of Garby, Sjölin & Vuille [ ], that the relative rate of synthesis of Hb F declines rapidly during this period of life.

About 50–75 days after the injection, the activity in HbF showed a definite tendency to decrease in all infants studied beyond this time. This must be taken as evidence that red-cell death already starts at this time, at least in those cells that contain foetal haemoglobin. The problem will be treated more extensively in a forthcoming publication [9].

The faecal excretion of iron. To the best of our knowledge no direct measurements of the daily excretion of iron in young infants have been reported. Josephs [11], in an attempt to calculate the need of iron, estimated the daily excretion to be of the order of 0.015 mg/kg/day. Smith *et al.* [10], who injected red cells labelled with Fe^{55} into pregnant women and followed the specific activity of the iron in the red cells of the offspring of these women up to 2½ years after birth, found that at * years, over 90 % of transplacental iron was still utilized for haemoglobin. This figure, which was arrived at by comparing the total activity in the circulation at birth with the total activity in the circulation at the age of 2 years, has been used by Betke [1] to estimate roughly the daily loss of iron. However, since little is known about the distribution kinetics of the labelled iron between the red cell compartment and other body compartments during this period of life, we do not feel that it is possible from these measurements to draw any conclusions concerning the rate of excretion of iron. In fact, if the same calculations are made at the age of one year, it is found that substantially more than 100 % (!) of the total activity at birth is present in the circulation at one year of age. This surprising figure probably reflects errors in the calculated blood volumes of these infants as well as net shifts of iron from stores to the red cells.

In adults, generally accepted figures for the daily excretion of iron are about 0.005–0.01 mg/kg in men and about twice that amount in menstruating women, although no direct measurements seem to have been made. The data obtained by Finch [3] are probably the best existing. Finch measured the rate of decrease in the radioactivity in circulating haemoglobin after an intravenous injection of Fe^{55}. He found that this rate did not approach a first-order reaction until 1–* years after injection, and interpreted the finding as indicating the presence of very slowly exchanging iron stores. The rate constant of the "final single" slope was used to calculate the rate of iron excretion. This rate constant must represent a maximum value since further gradual mixing with iron stores could not be excluded. Although there is some doubt as to the magnitude of the total miscible pool of iron that may be used in calculating the excretion rate of iron, it appears that the figures obtained by Finch, 0.01 mg/kg/day, represent maximum values for iron excretion in men and non-menstruating women.

Dubach, Moore & Callender [ ] measured the faecal excretion of radioiron after intravenous injection in adults, and

followed the excretion of the isotope for the following 140 days. The excretion of iron was then calculated by assuming that the specific activity of the excreted iron was identical to the value obtained by dividing th injected radioactivity by the estimated total body iron In view of the findings of Finch [3] this assumption is probably erroneous, and will lead to an overestimate of the daily excretion. The values for the daily excretion of iron thus calculated were close to those obtained by Finch, a fact that probably means that both estimates are too large

Our data for three infants seem to open up a new and from a theoretical point of view a more appropriate pproach to the problem of iron excretion. Let us consider that moment in the infant s lif when the haemoglobin concentration in the peri pheral blood has reached its minimum that is at about two months of age From this time on, the haemoglobin concentration remains relatively constant over a con siderable period the total haemoglobin mass increasing more or less proportion ately with the gain in body weight. From this increase and a daily haemoglobin destruction of about 1–2 % of the total haemoglobin mass (cf. Garby Sjölin & Vuille [9]), the daily haemoglobin produc tion can be predicted to be about 2.5 % of the total circulating haemoglobin mass. In our three cases, the period was characterized by a steady increase in the radio- ctivity in the circulating haemoglobin. From this increase and an assumed daily production of 2.5 % of the total haemoglobin mass, the specific activity of the bone-marrow iron precursor pool during this period can easily be calculated. Now at this stage of the experiment (between 30 and 60 days after injection) i.e before any significant destruction of labelled cells has begun, the alteration in specific activity in any iron pool as a function of time is negligible compared to the velocity of the iron-exchange between the plasma and the bone marrow pool(s) so that the assumption that the specific activities of the iron in the bone marrow and the plasma are almost equal cannot be far wrong It is also probable that the precursor iron for excretion into the bowel will have a specific activity very close to that of the plasma The amount of iron excreted (E) can then be calculated thus,

$$E = R/SA$$

where R is the amount of excreted radioactivity per unit of time and SA the speci fic activity calculated as indicated above

The values obtained from these calcula tions in the three infants Bl, Sj and Ja were 0 14 0 16, and 0 13 mg/day or 0 032, 0.036 and 0.026 mg/kg/day respectively The possible error introduced by the estimation of the daily haemoglobin syn thesis and by the assumption concerning the specific activity of the precursor iron would presumably not suffice to alter these figures by more than a factor of 2. A com parison with the values for normal adults would therefore suggest that young infants lose three to six times as much of their iron as adults. Further our results provide still more evidence in favour of this hypothesis At about the 95th day after injection, an unmistakable peak appeared in the excretion of radioiron (about 5–10 times as much as during the preceding period). This peak is undoubtedly connected with the death of labelled cells and it is significant to note that it was not observed in

the experiments in adults carried out by Dubach Moore & Callender [9] The high rate of iron excretion in this age group is of interest in view of the findings of Garby & Sjölin [5] that the absorption of labelled iron in the same age group was considerably greater than in adults. Thu the exchange of iron with the environment would seem to be much greater in infant than in adult

The terminology associated with the study of transfer of iron across the intestinal mucosal barrier has been used somewhat loosely. The reason for th is to be found in incomplete knowledge of what is actually measured in a tracer experiment. The f llowing discussion i designed to show that thi question is not only of academic interest

Since w have no detailed knowledge concerning the mechanisms of the movement of iron across the mucosal barrier w ha e to confine our argument to the general case in which there i a conti uous flow of iron in both directions across the barrier. From the standpoi t of tracer methodology it would appear reasonable to assign the word *absorption* t the sum of ll flows in the direct on from the testinal l men to the body proper and the word *xcretion* to the sum f all flows i the opposit lrection. It hould be stressed however that such a terminology would *not* be in harmony w th the general clinical usage f these words. For instance f a tracer experiment ha shown that 10 of a given preparation of labelled ron (containing non labelled iron atom as well) under given conditions taken p by the body during the passage through the ntestines, t follow that 10 f the non labelled tom will lso ha e been taken p. Thus, from the tracer methodological standpoint it may be stated that the 'absorption f iron n ler these conditions is 10 %. In this sense however the term absorption is not equi alent to the usual meaning f the word, which at any rat m the case of iron, is equivalent to that part of the gi en dose that is *reta ned with n the body for a reasonable t me*. In the case f iron a reasonable time is of the order of weeks or months. The tracer xperiment referred to bove gi es no information concerning the net transfer f iron *from the env ronment i.e. the intestines, to the body proper* ince th "excretion remain unknown. *It should be pointed out* that the coupling between absorption and "excretion" of iron may be quit close In order to gi e a concrete xample let us consider the extreme case where the migration of iron across the barrier takes place as an ex hange tom for atom (cf the link d-impermeabl e" species l scussed by Patlak [14]). Here the absorption i momentaneously f llowed by an equally great "excretion and although a tracer xperiment would indicate a finite absorption of iron, there i no retention of iron in tl i case

Dubach Moore & Callender [ ] discu sed the possible mechanism of iron excretion by the bowel. They concluded that the bile and cells desquamated or lost into the bowel provide at least some of the *iron in the faeces* and they believe that active excretion b the bowel is much less likely. It is possible that a significant part of excreted iron in young infant i derived from red cell lost by diapedesis or micro-scopic bleeding" and Hong Wallerstein & Pollycove [10] have presented some evidence that this may indeed be the case. The peaks of radioactivity excretion in connexion *with the destruction of labelled* cell in our experiment provide strong evidence that most of the excreted iron consists of non red-cell iron however

Summary

Long term studies on five infant in whom radioactive iron had been injected intravenously provide further support to the earlier statement that the rate of syn

thesis of haemoglobin is relatively very low after the first few days and up to 1½–2½ months of life: after that the activity of the bone marrow again increases.

Further the data reported are in accordance with results of previous investigations concerning the relative rate of synthesis of haemoglobin F and haemoglobin A, showing that during the first weeks of life there is a rapid decrease in the relative rate of HbF production.

The findings in this study indicate further that the destruction of red cells produced during the newborn period already starts after about 60–70 days.

The faecal excretion of radioiron was measured in three cases. Some theoretical considerations concerning the calculation of iron excretion from tracer data are given, and a figure for the daily iron excretion of about 0.03 mg/kg is suggested in these infants. This estimate is three to six times that of the usually accepted figure in adults.

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the experiments in adults carried out by Dubach Moore & Callender [ ]. The high rate of iron excretion in this age group is of interest in view of the findings of Garby & Sjölin [5] that the absorption of labelled iron in the same age group was considerably greater than in adults. Thus the exchange of iron with the environment would seem to be much greater in infants than in adult.

The terminology associated with the study of transfer of iron across the intestinal mucosal barrier has been used somewhat loosely. The reason for this is to be found in incomplete knowledge of what is actually measured in a tracer experiment. The following discussion is designed to show that this question is not only of academic interest.

Since we have no detailed knowledge concerning the mechanisms of the movement of iron across the mucosal barrier we have to confine our argument to the general case in which there is a continuous flow of iron in both directions across the barrier. From the standpoint of tracer methodology it would appear reasonable to assign the word *absorption* to the sum of all flow in the direction from the intestinal lumen to the body proper and the word *excretion* to the sum of all flow in the opposite direction. It should be stressed however that such a terminology would *not* be in harmony with the general clinical usage of these words. For instance if a tracer experiment has shown that 10 % of a given preparation of labelled iron (containing non labelled iron atoms as well) under given conditions is taken up by the body during the passage through the intestines, it follows that 10 % of the non labelled atoms will also have been taken up. Thus from the tracer methodological standpoint it may be stated that the absorption of iron under these conditions is 10 %. In this sense however the term absorption is not equivalent to the usual meaning of the word, which, at any rate in the case of iron, is equivalent to that part of the given dose that *is retained with the body for a reasonable time*. In the case of iron, a "reasonable time" is of the order of weeks or months. The tracer experiment referred to above gives no information concerning the net transfer of iron from the environment i.e. the intestines, to the body proper since the excretion remains unknown. It should be pointed out that the coupling between absorption and excretion of iron may be quite close. In order to give a concrete example let us consider the extreme case where the migration of iron across the barrier takes place as an exchange atom for atom (cf. the "link d-impermeative" species discussed by Patlak [14]). Here the absorption is momentaneously followed by an equally great excretion and although a tracer experiment would indicate a finite absorption of iron, there is no retention of iron in this case.

Dubach Moore & Callender [ ] discussed the possible mechanisms of iron excretion by the bowel. They concluded that the bile and cells desquamated or lost into the bowel provide at least some of the iron in the faeces, and they believe that active excretion by the bowel is much less likely. It is possible that a significant part of excreted iron in young infants is derived from red cells lost by diapedesis or microscopic bleeding" and Hoag Wallerstein & Pollycove [10] have presented some evidence that this may indeed be the case. The peaks of radioactivity excretion in connexion with the destruction of labelled cells in our experiment provide strong evidence that most of the excreted iron consists of non red-cell iron however.

Summary

Long term studies on five infants in whom radioactive iron had been injected intravenously provide further support to the earlier statement that the rate of syn

TABLE 1 *Levels of dietary supplementation*

	Dosage levels for							
Vitamin, mg/day	1st month (I)	2nd month (II)	3rd month (III)	4th month (IV)	5th month (V)	6th month (VI)	th month (VII)	8th month (VIII)
Ascorbic acid	4.0	8.0	16.0	35.0	60.0	100.0	150.0	200.0
Thiamine	0.2	0.4	0.8	1.5	3.0	5.0	10.0	20.0
Riboflavin	0.10	0.2	0.4	0.8	1.5	3.0	6.0	10.0
Pantothenic acid	[illegible]	4.0	6.0	8.0	15.0	20.0	30.0	50.0
Nicotinic acid	2.0	4.0	8.0	10.0	15.0	20.0	30.0	60.0
Cyanocobalamin	0.001	0.002	0.004	0.010	0.020	0.030	0.100	0.200
Biotin	0.010	0.020	0.040	0.060	0.100	0.150	0.200	0.250
Pyridoxine	0.4	0.8	1.5	3.0	5.0	10.0	[illegible]0.0	40.0
Folic acid	0.20	0.4	0.8	1.0	1.5	3.0	5.0	10.0

The subjects were divided into two groups of five each, an experimental group receiving vitamin supplementation, and a control group receiving no supplementation, the two groups being matched with regard to initial dietary intake and found to be matched with regard to milk constitution.

Supplementation procedure

The supplementation was done simultaneously with regard to the vitamins studied previously viz., ascorbic acid, thiamine, riboflavin, panthothenic acid, nicotinic acid, cyanocobalamin, biotin, pyridoxine and folic acid. The dosage levels were increased progressively from initially low values to recommended levels of intake and beyond, each level being maintained for month. Eight such levels were used as shown in Table 1 Synthetic preparations of the vitamins were used and appropriate amounts of the same were administered in the form of a solution except for ascorbic acid which was given in powder form. The subject was required to take the supplement in the presence of the experimenter

Collection and analysis of milk

Collection and analysis of milk were done according to the procedures described elsewhere [5]. The analyses of milk was done at the end of each supplementation treatment prior to the introduction of the next dosage level.

Estimation of milk yield

The 24 hrs intake of the infant was taken as the total yield of milk and was determined by weighing the infant before and after each feed.

Results

The changes in the milk levels of the vitamins with the progress of supplementation are shown in Table [illegible] from which it can be seen that the vitamin content of milk increases steadily with the dose of supplementation, significant increases being observed in many cases by the first month of supplementation, and in any case by the third month. However although the values continue to register an increase throughout the period of supplementation, the increments in milk levels are seen to diminish towards the end of the supplementation period.

Table 3 shows the initial and final values obtained in this study in comparison with the maximum values reported by other

(The rat group received no supplementation)

Vitamin		Level prior to supplementation	I	II	III	IV	V	VI	VII	VIII
			Dosage level							
Ascorbic acid mg/100 ml	Experimental	_3 ±0.1	_4 0	2.8 0	2.0 0._	2.4 0._	2.8 _0.2	4.0 ±0.3	5.4 ±0.3	6.1 ±0.3
	Control	_4 0	_4 0.2	2.6 _0._	_4 _0._	_5 +0._	_5 0.2	_5 _0._	2.5 ±0.2	_4 ±0._
Thiamine mcg/100 ml	Experimental	10.9 +0.1	12.5 0.2	13.4 0.3	14.2 _0.4	16.0 _0.4	18.3 ±0.1	_2_ _0.5	25._ ±0.5	_8.8 ±0.7
	Control	10.9 0.2	11.3 0.2	11.6 0.3	12.1 ±0.2	1_.0 ±0.3	11.3 ±0.2	11.6 _0._	10.8 _0.2	1__ _0._
Riboflavin mcg/100 ml	Experimental	_0.0 _0.5	_2.0 0.5	_6.0 +1.6	9.0 ±1.2	31.0 ±1.1	45.0 ±1.0	57.0 ±1.9	67.0 ±_8	74.0 ±_9
	Control	_0.0 0.5	1.0 +0.6	_2.0 +0.6	_2.0 _0.5	_2.0 ±0.6	22.0 ±0.6	4.0 ±0.5	21.0 _0.6	_2.0 ±0.8
Pantothenic acid mcg/100 ml	Experimental	100.0 1.6	130.0 1.9	142.0 ±2.0	16_.0 ±3.3	193.0 _4.0	_35.0 ±4.2	_71.0 ±5.0	240.0 ±5.9	302.0 ±8.2
	Control	88.0 11	102.0 _1.1	104.0 1.5	104.0 ±1.6	107.0 _1.4	105.0 ±1.8	104.0 _1.5	107.0 ±1.2	102.0 ±1.3
Nicotinic acid mcg/100 ml	Experimental	98.0 1.5	117.0 _2	1_8.0 2.0	133.0 _2.7	153.0 ±4.8	14_ +6.7	18_._ ±7.4	_84.0 ±7.3	275.0 ±8.8
	Control	98.0 1	101.0 _8	101.6 _2	101.0 ±_6	103.0 ±_4	103.0 ±__	102.0 ±_7	102.0 ±_5	102.0 ±_6
Cyanocobalamin mcg/lit	Experimental	0.078 +0.001	0.07_ ±0.001	0.080 ±0.002	0.08 ±0.002	0.081 ±0.003	0.086 ±0.003	0.088 ±0.002	0.096 ±0.003	0.100 ±0.001
	Control	0.077 _0.00_	0.07_ ±0.002	0.078 ±0.00_	0.07_ ±0.002	0.077 ±0.002	0.078 ±0.002	0.078 ±0.002	0.0_8 ±0.002	0.077 ±0.002
Biotin mcg/100 ml	Experimental	0.16 ±0.01	0.18 ±0.01	0.20 ±0.03	0.__ ±0.03	0.25 ±0.03	0.38 ±0.03	0.42 ±0.03	0.45 ±0.01	0.50 ±0.03
	Control	0.15 ±0.01	0.15 ±0.01	0.16 ±0.01	0.16 ±0.01	0.16 ±0.01	0.16 ±0.01	0.16 ±0.01	0.16 ±0.01	0.16 ±0.01
Pyridoxine mcg/100 ml	Experimental	8.0 ±0._	8.4 ±0.2	8.9 ±0.3	9.5 ±0.2	10.7 ±0.3	12.2 ±0.3	14.1 ±0.3	15.5 ±0.4	15.8 ±0.3
	Control	7.8 ±0.2	7.8 ±0.2	7.7 ±0.2	8.0 ±0._	8.5 ±0.2	8.6 ±0.2	8.3 _0.2	8.2 ±0.2	8.0 ±0.2
Folic Acid mcg/100 ml	Experimental	0._0 ±0.02	0.23 _0.02	0._6 ±0.0_	0.27 ±0.02	0.30 ±0.03	0.36 ±0.03	0.40 ±0.03	0.5_ ±0.03	0.54 ±0.04
	Control	0.19 ±0.02	0.20 ±0.02	0._0 ±0.02	0.21 ±0.02	0.21 ±0.02	0.22 ±0.02	0.21 ±0.02	0.21 ± .02	0.21 ±0.02

The values were determined at the end of the supplementation period. The levels of supplementation were increased progressively from the 1st to the 8th month.

TABLE 3 *Comparative values for the vitamin levels of milk with and without supplementation*

Vitamin	With supplementation: Present study: Initial	Present study: Final	Other studies: Initial	Other studies: Final	Without supplementation (Maximum value reported)
Ascorbic acid mg/100 ml	2.3	6.1	2.8	3.6	4.4 [13]
Thiamine mcg/100 ml	10.9	26.8	11	25	19.4 [7]
Riboflavin mcg/100 ml	20.0	74.0	28	48	40.9 [13]
Pantothenic acid mcg/100 ml	100.0	303	220	300	223 [9]
Nicotinic acid mcg/100 ml	98.0	275	9.2 ppm	18.4 ppm	173 [13]
Cyanocobalamin mcg/lit	0.078	0.100	.06–.2	.35–60	0.41 [4]
Biotin mcg/100 ml	0.16	0.50	—	—	0.35 [13]
Pyridoxine mcg/100 ml	8.0	15.6	13.5	100. 400	18.0 [10]

investigators with and without supplementation. It can be seen from the same that although the initial values are far less than those reported by other investigators, ranging from 20% to 60% of the latter the final values, except for cyanocobalamin, compare favourably with the values obtained by other investigators after large doses of supplementation In the case of cyanocobalamin, however in spite of prolonged supplementation far above the recommended level of intake, the milk level fails to come up to the generally reported level which contrasts with the immediate increase from 0.06 to 0.20 reported by Karlin [8] However this vitamin was administered orally without the intrinsic factor and we can only presume that its effectiveness might have been reduced thereby

The 24 hrs yield of milk during the 5th month of supplementation and the estimated secretion of vitamins during this period are shown in T ble 4 long with infant requirements It can be seen that, while vitamin supplementation has a salutary effect on milk yield the amounts of vitamins available to the infant are still short of the recommended levels except with regard to ascorbic acid This would appear to be so because there is no concomitant increase in milk yield. However the gap between requirement and availability is found to be considerably narrowed and might be expected to have been further narrowed at the higher levels of supplementation used.

Discussion

Thus the present studies confirm previous findings that the administration of oral vitamin supplements to lactating women has beneficial effects of vitamin levels of milk, and further suggest that in subjects with low levels of vitamin intake beneficial effects may be obtained by relatively small doses of supplementation for prolonged periods.

Vitamin requirements during lactation have not been determined with regard to several vitamins Moreover the same may

TABLE 4 *Twenty four hours milk yield and vitamin content after four months of supplementation*

	Experimental	Control (No supplementation)	Recommended allowance for infants
Twenty four hours milk yield ml	600.0 ± 15.0	550.0 ± 17.0	
Ascorbic acid mg	22.6	13.8	30.0
Thiamine mg	0.11	0.06	0.4
Riboflavin, mg	0.[illegible]7	0.1	0.6
Pantothenic acid mg	1.41	0.58	—
Nicotinic acid, mg	1.[illegible]	0.56	4.0
Cyanocobalamin, mcg	0.05	0.013	—
Biotin, mcg	[illegible]29	0.88	—
Pyridoxine mg	0.073	0.047	—
Folic acid, mcg	[illegible]10	1.20	—

The supplementation was carried out in progressively increasing doses as shown in Table 1

depend on the prior nutritional status of the subject In this context Gopalan [6] has suggested the minimum maternal intake supporting maximum milk concentration as a possible criterion

From this point of view it would be of interest to compare the dosage corresponding to ceiling milk levels with the recommended allowances Such a comparison is made in Table 5 which also gives the dosage at which the maximum values reported by other investigators are reached In regard to many of the vitamins the dosage corresponding to ceiling levels is *seen to be considerably greater than* recommended level However the difference has to be considered in relation to the initial low nutritional status of the subjects. It would also appear from the table that the dietary inadequacy with regard to thiamine pyridoxine and folic acid is relatively greater To this list must be added cyanocobalamin on the basis of its low level in milk

An interesting observation emerges with regard to the ascorbic acid secretion in milk. Although a high correlation has been found by us between the dietary intake and milk constitution of this vitamin a scrutiny of the data for the control group reveals that the amount secreted in milk is of the order of 14 mg which is far in excess of the dietary intake (1.5 mg) of this in group which was arrived at by the actual analysis of the diet consumed for consecutive days Although some seasonal variation in dietary intake cannot be ruled out it is to be noted that steady milk levels are maintained throughout the period studied This intriguing observation prompted a re-analysis of the results of the *cross-sectional study previously reported* [5]. On a conservative estimation of a milk yield of 500 ml a day the intake and milk secretion of ascorbic acid in different ranges of intake are shown below

Quartile Range	Ascorbic acid (mg) Mean intake	Estimated milk secretion
I	0.6	12.00
II	2.4	12.50
III	4.4	16.00
IV	8.5	23.0

The discrepancy found in the above data between dietary intake and milk secretion is intriguing since ascorbic acid is not known to be stored in appreciable amounts in the body

A similar scrutiny of the data with regard to the other vitamins studied showed the vitamin secretion in milk to be well within the amounts consumed in the diet Although the method used for the estimation of ascorbic acid does not measure bound ascorbic acid either in diet or milk, this omission can hardly be considered to account for the gross disparity between milk and diet values.

Although the blood level of ascorbic acid [11 14 16] has been found to drop after parturition, and not to return to normal levels till after the termination of lactation [11] on the basis of total blood volume, it is unlikely that this fall can account for the estimated excess secretion of more than 2000 mg of ascorbic acid during six months of lactation. The hypothesis of tissue depletion to this extent also does not appear plausible in view of the fact that about *000 mg are found to be needed to achieve tissue saturation in scorbutic patients [15] so that depletion to this extent after meeting maternal requirements may be expected to result in severe scorbutic symptoms. In this connection, certain data reported by Snelling & Jackson[16] yield a mean ascorbic acid content of 3.5 mg % for subjects with low blood levels of the vitamin. Bagchi [2] and Bharachua [3] have found middle class infants with low maternal intake of ascorbic acid to be free from scorbutic symptoms. Such observations have led some investigators to raise the question as to whether there is any endogenous production, particularly during lactation [1 – 12]. The above observations underline the need for further studies on ascorbic acid metabolism in the human body particularly during lactation.

In conclusion, the results of this experiment demonstrate the beneficial effects of vitamin supplementation of milk vitamins, and to some degree on yield as well, even when the supplementation is commenced at small doses. It would thus ppear that the results obtained in such studies may be influenced not only by the absolute levels of supplementation, but also by the prior nutritional status of the subject.

Summary

Oral vitamin supplementation was administered to a group of five lactating women whose diets were grossly inadequate with regard to vitamins, fat, and protein. Another group of five subjects of like nutritional status served as controls

The supplementation was commenced at low levels and increased gradually to recommended levels and further Eight such levels were used, each level being maintained for one month. The vitamins supplemented were ascorbic acid, nicotinic acid riboflavin, thiamine pantothenic acid cyanocobalamin, biotin, pyridoxine and folic acid.

Beneficial ffects on milk levels were obtained within the third month of supplementation.

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Detection of Enteropathogenic Escherichia Coli in a Swedish Watercourse (the River Fyris) by Means of Fluorescent Antibodies and by Conventional Methods

by DAN DANIELSSON and GUNNAR LAURELL

After the basic work by Bray [1] and Taylor *et al.* [17] there are now a large number of E. coli serotypes considered to be associated with epidemic infantile diarrhoea. They are most frequently isolated in connection with epidemic outbreaks in hospitals or children s homes, but they are also found in children who fall ill outside a hospital. The ways by which the illness is spread from case to case are well known [10 15] but less is known about the extra-human occurrence of the serotypes and about possible reservoirs of infection. Recently Ewing [7] has issued a report covering an 11 year period from 1950–1960 of the sources of E. coli cultures belonging to serotypes associated with infantile diarrhoea. Most of these were isolated from faeces originating from humans and animals with diarrhoea, but strains were also isolated from extra intestinal sources, viz cheese tap water and milk. The occurrence of enteropathogenic E. coli in specimens of non faecal origin has been reported by Jones [8]. In France E coli 026B6, 055B5 and 0111 B4 have been isolated from different drinking water sources by Monnet *et al* [11] and Seigneurin *et al* [16] and in a mountain stream in the Rocky Mountain area 086B1 and 011 ae have been isolated by Petersen *et al* [13]

In the autumn of 1961 we took a water sample from the river Fyris in Uppsala. In this sample we could identify enteropathogenic E. coli by the direct fluorescent antibody technique and this finding was verified by the conventional cultivation method. In consequence we continued to examine water samples from this river during a 6-month period (November 1961–May 1962) The enteropathogenic E. coli found in this investigation have been reported in the previous work [3]

Material

Water samples were collected from the Fyris at the so-called Islandsbron in Uppsala. The Fyris originates from a small lake in the north of Uppland, collects water from smaller streams, passes through Uppsala and falls into Lake Mälaren. The sampling point is situated about 1 6 km above the outlet of the water from the sewage treatment plant and about 8 km from the outflow f the Fyris int Lake Malaren.

Water samples were collected in a sterile glass bottle and were subjected to investigation within one hour after collection.

Method

(I) *Estimation of the coliform density*

The standard multitube method (MPN method) was used for the estimation of the coliform density Lactose peptone broth with sodium formiat wa used as medium, and to the series of tubes set up water was added in the following way: five 10 ml, five 1 ml and f 0.1 ml. Because f the high degree of contamination with coliform organisms the water samples were as a rule lluted 1/10 and sometimes 1/100 The tubes were incubated at 3° C and were read off after an incubation time of 48 hours. The estimation was done only as a presumptive test and gas prod iction in a tube was con sidered to indicat the presence of coliform organisms.

(II) *Production of antisera*

Young albino ral bits weighing 2 .5 kg were used. Immunization was carried out according to the procedure laid down by Edward & Ewing [6]. Antisera were produced against the following E. coli strains: 0*6B6, 0.5B5, 0*6B7 0111B4 011 b and ac 0114B? 0119B14 0125B15, 01*6B 16, 01*7B8, 0128B1. The titres of the sera were est mated by the current tube agg lutination technique In acceptable sera the O-titres w re as a rul 1 1 80-51*0 and the B-t tres 1 1 8 256

(III) *Conjugation with fluorescent substance*

The globulin portion f each antisera was conjugated with fluorescein isothiocyanat (abbreviated FITC) according to procedures described in a previous paper [4] or with Lissamine rhodamine B*00 (abbreviated RB*00) in the manner described by \ rn [12]. Unconjugated substance was removed by passing the conjugate through a column packed with Sephadex G-.5 (Pharmacia, Uppsala) in phosphate-buffered physiological saline pH 7.2

The staining titre of each of the anticoli globulin conjugates was estimated in the manner described before [4]. They gave as rule good staining reactions (judged as 3-4 +) at d lutions of 1:16-1:32

The specificity of the conjugates was investigated by testing them against heterolog as enteropathogenic E. coli strains, nonenteropathogenic coli strains, strains belonging to Pseudomonas aeruginosa, Aerobacter aerogenes, and Proteus. When undiluted the conjugat of anti 0112ab, gave a weak cross reaction (judged as 1 2+) to 011 ac and the conjugate of anti 0127B8 to 086B7 These cross reacti ns were how ever no longer isibl at a dilution of 1:10. To the other strains tested the conjugates did not show any cross reactions.

(IV) *Preparation and staining of smears*

One ml broth wa taken from those tubes in the MI N-specimen exhibiting growth and the production of gas. The broth was pooled from each series of tubes and centrifuged at 3000 rpm for *0 minutes. The sediment was suspended in physiological saline and smears were made f r treatment with fluorescent antibodies. Staining was performed in the usual manner [4]. To begin with tw smears were tested with pooled conjugates, each consisting of six anticoli glob lin conjugates. Three of these were conjugated with FITC and three with RB*00 Bacteria reacting to FITC-conjugates ppeared with a yellow green colour in the fluorescence microscope and those reacting to RB*00 conjugates appeared with an orange colour If a positiv result was obtained we tested smears with single globulin conjugates from the different pools to rrive at a definit diagnosis. Con trol test were made on normal rabbit globu lin and also by means of inhibition tests.

(V) *Fluorescence microscopy*

A Zeiss fluorescence microscope equipped with a dark field condensor and an Osram HBO*00 mercury lamp was used. A BG12

(3 mm) was used as primary filter and a Zeiss 47 or a Zeiss 50 as secondary filter. The following scale was used for reading of the number of fluorescent bacteria.

Negative: No fluorescent bacteria present.

Infrequent (I): Single fluorescent bacterium per field.

Moderat (M): 2–50 fluorescent bacteria per field.

Abundant (A): More than 50 fluorescent bacteria per field.

(VI) *Isolation of enteropathogenic E. coli*

For the isolation according to conventional methods we used Conradi-Drigalski blue agar plates [9]. We first tried with direct inoculation of crude river water but with negative results. The same sediment as was used for preparation of smears for the fluorescent technique was then used for inoculation of the plates. After incubation at 37° for 20–24 hours about 40–50 colonies were examined by means of slide agglutination tests. Two pooled antisera were used, each pool containing six antisera. Colonies giving a positive agglutination were cultured on a glucose agar plate and then tested with single sera to determine the K-antigen. The 0-antigen was confirmed by the current tube agglutination technique after the growth of positive colonies in broth and boiling at 100°C for 90 minutes. Suspected strains were also tested biochemically using lactose, sucrose, inosite, adonite, mannitol, tryptophan and urea. This biochemical testing was of great value especially with strains belonging to 0112 b and 0112ac.

Result

1. *The coliform density*

The results of the MPN estimation of the coliform density are presented in Table 1.

The coliform density did not show much variation from the middle of November 1961 when the investigation started, till the end of March 1962. Then, however, a

TABLE 1. *The results of the MPN estimation of the coliform density*

Sample no.	Month and day	Coliforms/100 ml
1	Nov. 20th	1,500
2	Dec. 11th	1,800
3	Dec. 18 th	1,600
4	Jan. 2nd	1,700
5	Jan. 8th	1,800
6	Febr. 14th	1,500
7	March 29th	1,500
8	Apr. 3rd	1,500
9	Apr. 10th	20,000
10	Apr. 24th	7,000
11	Apr. 31st	3,000
12	May 14th	1,500

pronounced increase was noted, the content of bacteria increased tenfold in one week and continued to increase during the following week when it mounted to 20 000 coliforms per 100 ml. The highest values were recorded after the breaking up of the ice and the thawing of the snow, a process which culminated in the middle of March. The water level in the Fyris rose by about 1 metre as a result of this and the pollution of the water was very pronounced. Two weeks later the coliform density was 7000 and in May it was 1500/100 ml, i.e. about the same as before the break up of the ice.

2. *Identification of enteropathogenic E. coli by means of fluorescent antibodies and isolation by conventional methods*

Fig. 1 provides an illustration of the double staining technique employed. Anticoliglobulin 055B5 is here conjugated with FITC and these bacteria in consequence give a green fluorescence while anticoliglobulin 0111B4 is conjugated with RB200, the bacteria in consequence giving an orange fluorescence.

TABLE 2 *Results obtained by the conventional method and by the fluorescent antibody technique together with the quantitative grading*

Sample no.	Month and day	Isolation by con entional method	Identification by fluorescent antibodies A	M	I
1	Nov 20th	055 0112 b	011 b	055	
2	Dec 11th	011 ac		0112 ac 0125	0127
3	Dec 18th	01_5	01_5		011 ac 0127
4	Jan 2nd				011 b, 0125
5	Jan. 8th				0114 0119
6	Febr 14th				055, 0125
7	March 20th	01_5	0125		026, 0112 ac 0114 0119, 0129
8	Apr 3rd	0127	0127		086, 0112 ab, 0119 0125 0126, 0128
9	Apr 10th	0119	0119		086, 0112 ac 0114 0125 0126, 0127
10	Apr 4th	0119	0119	011 ab 0112 ac	086, 0114
11	Apr 21 t			0119	055, 0114 01_5
12	M y 14th	0114	0114	01 8	055, 086

Table 2 records the general findings concerning the occurrence of enteropathogenic E coli during the investiga ion.

As the table hows, 48 strains belonging to ten different 0 groups were identified by means of the fluorescent antibody technique Most of them occurred in frequently (I) but sometimes they were found in moderate (M) or abundant (A) numbers. It should however be borne in mind that the identification technique employed involved an enriching The highest number of strains wa identified when the coliform density was high i e after the ice had broken up Seven of the strains were identified on four to eight different occasions Most of the strains which were identified belonged to types which had not caused epidemics in Sweden, but had only been recorded in connection with individual cases of diarrhoea.

By means of the conventional technique nine strains belonging to six different 0 groups were isolated Three strains were isolated on two separate occasions. When strain were isolated by means of conventional culture they could always be demonstrated by the fluorescent antibody technique

Discussion

The investigation of a minor water course in Sweden by means of the direct fluorescent antibody technique resulted in the identification of 48 enteropathogenic E. coli in all on 1* different occa sions. The conventional technique in

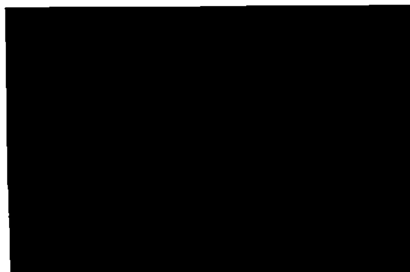


Fig. 1 An example of the double staining technique (see text). 055 B 5 – green rods. 0111 B 4 – orange rods. Film: Super Anscochrome, 35 mm, Tungsten. Exposure: 70 sec.

contrast only recorded nine strains on the same occasions. The fluorescent antibody method thus showed a significantly greater degree of sensitivity The method of cultivation used here in the investigation of the bacterial sediment from a tube of broth in a normal presumptive water test should, to judge by Dixon s experiments [5] be considered to be a particular form f enriching He has shown that when two or more coliform strains are inoculated into nutrient broth in unequal numbers incubation at either 37° or 44 C results in an increase in the proportion of the more scanty strain.

When judging the results obtained with the fluorescent antibody technique it must be remembered that the method is strictly serological and that cross reactions with other bacteria may give false positive results. All things considered however this seems not to have interfered with the diagnosis. Thus, for example the presence of a serotype could always be verified by the conventional method if its presence was classified as abundant by the fluorescent antibody method. Neither did the conjugates used in this investigation show any cross reactions to bacteria so far tested. Moreover it has been hown in a previous paper [3] that after the enrichment of a specimen the sensitivity of the fluorescent antibody technique is increased as compared with the conventional method.

The pathogenic serotypes occurred most plentifully when there was high density of coliforms in the watercourse i.e in the spring after the thawing of the snow and the breaking up of the ice Most of the serotypes isolated have not been associated with epidemics in our country but they have been isolated from individual cases of infantile diarrhoea The origin of the pathogenic serotypes is obscure but the increased frequency during the spring and after the break-up of the ice points to bacterial pollution from cultivated soil. Cattle may be of some importance a supposition which is supported by the fact that serotypes 086B 0114 and 0119B14 have been isolated from calves [2, 14 18 19]; we isolated 0114 once and 0119B14 twice in spring The present investigation shows in any case that the enteropathogenic serotypes of E. coli are widely distributed outside the human organism and that they can occur plentifully in polluted watercourses

Summary

During a 6-month period (November 1961–May 1962) a minor watercourse in Sweden (the river Fyris) has been investigated by the fluorescent antibody technique and by conventional method on the occurrence of Escherichia coli serotypes associated with infantile diarrhoea Forty-eight strains belonging to ten different 0 groups were identified by fluorescent antibodies By conventional technique nine strains belonging to six 0 groups were isolated. The origin of the strains is discussed.

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CASE REPORT

Congenital Agammaglobulinaemia in the Brother of a Boy who Died of Generalized BCG Infection

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At the Department of Paediatrics Uppsala in January 1962 a boy died of general sepsis with signs of congenital agamma globulinaemia. A brother of this child had died in 1953 of generalized BCG infection [5] The occurrence of these two, both rare illnesses in two brothers in itself justi fies publication. As will be apparent from the following there is evidence that the boy that died of BCG infection also had gammaglobulinaemia

Case history

Family history The patient had many relatives both on the father and the mother side, but, as fa as is known, none had showed any marked proneness t severe infections.

Sib 1 Boy born May 25, 1952 Birth weight 2820 g Died at 8 months of age of generalized BCG infection [5].

BCG vaccination was carried out in the usual way (intradermal injection in the left thigh) at the Department of Obstetrics when the infant was 5 days old. At the age of 6 months he developed lymphadenitis in the left groin, at 7 months he developed pyrexia which settled during penicillin therapy and cough, which persisted. He was admitted to the Paediatric Department t 8 months of age in very poor condition, dyspnoeic cyanotic, with an area of necrosis 20 mm in diameter at the site of vaccination and lymph nodes with the size of hazelnuts in the left groin and active rickets. X-ray examination of the chest disclosed a large opacity first in the upper lobe of the left lung, and later also in the right lung The whitecell count was 6900, with 22.5 % lymphocytes but no plasma cells. The Mantoux test (1 mg) was negative Despite treatment with large doses of tetracycline and blood transfusions, the patient died 12 days after admission. Postmortem examination revealed generalized t berculosis, and the bacteria that were demonstrated could not, even on detailed investigation, be distinguished from BCG

Sib. 2 A girl, born in 1957 She is healthy and not susceptible to infections.

Sib 3. Our patient. T E. a boy born July 14, 1961 Record no. 91/1962, Paed. Clin., Uppsala. Birth weight 3480 g

Owing to the brother illness, BCG vacci nation was not done The infant received breast milk and supplementary feeding until 4 months of age Nov 25, 1961 he contracted common cold, having been infected by his sister who quickly recovered. He was treated with nose drops and cough medicine from Dec. 15, 1961 also with penicillin, and later with tetracycline and chloromycetin (see Fig. 1). After one week at the Hospital for Infectious Diseases he was transferred

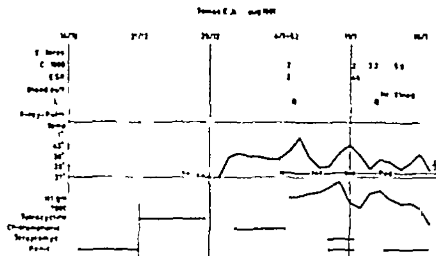


Fig. 1. Course of disease and treatment. X indicates examinations for which results are given in the text.

on Jan. 11, 196 t the Department of ardiat ics.

On adm n the patient had high pyrexia, b t was relativ ly little affected. There w no cyanosis or oedema, and no enlargement of ton il or other lymphoid tissue w noted. Ph ical xa nation of the chest showed fine rales on both sides. The respiratory rat was 100/minut. The heart sound were normal. The liver wa f lt 4 cm below the cost l margin. The spleen w not palpable. There were no l normal f nd ngs n the central n rvous sys tem.

X ray on the chest disclosed small cloud like opac ties the lower lobe f the left l ng. The ECG w normal, and the M n toux test (0.1 mg) negat

Laboratory test Haemoglobin 10.6 g/100 ml, there w anisocytosis nd poik locytosis in the red cell. Wh tecell cou t w 4900 (non-segmented neutroph l 0 polymorphonuclears 70 eosinoph l 0 basophil 0 lymphocytes 8 monocytes 2). Later whitecell count of 6900 7700 were recorded with increased relat ve lymphocytos. The micro-ESR was 4 51 44 mm. Bonemarrow biopsy gave a very small yield. No plasma cells could be demonstrated.

Test of l er f nction were normal. There wa no gl cosuria or album ria. The urinary sediment showed on one occasion 8 10 leucocytes per field but cult re yielded less than 1000 bacteria pe ml. In the faeces cult re no pathogenic bacteriae were observed but adenov ru type w isolated. Lumbar p ncture and cult re of the cerebrospinal fl d were normal a w the blood culture on two occasions. Guineapi test and Löwenstein cult re of gastric w hings for TB were negativ.

Serological investigations Ant staph lol si w 0.25 un t ml and ant treptolysin 04 nit per ml, coldagglutination w negative; ne tralization titre against adenovirus acut and con lescent serum w 1/16; complement f xation agai t adenovirus 1/3 and against Herpes imple 1 (acute and con alescent sera equal); neutralization titre against Coxsackie B_5 ~1/160, and against Coxsacki B 1/80 (acut and con alescent sera equal).

Isohaemaggl t ns The patient belonged to blood-group A Rh( ). Only traces of anti B could be demonstrated, using spe cially sensit e method [4].

Complement The activities of tot l complement and of the component C C_1 C_2

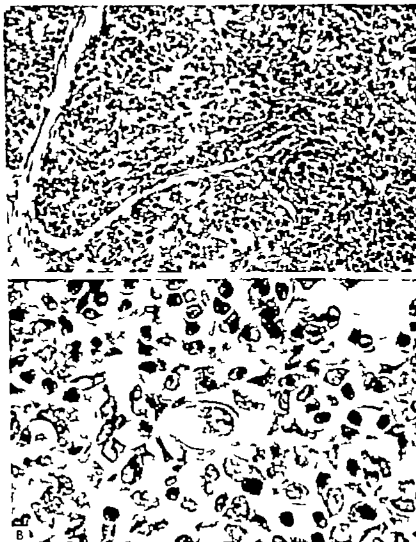


Fig 2A. Section from the thymus. There is pronounced lymphoid hypoplasia with only sparse lymphocytes and marked overgrowth of hyperplastic reticuloepithelial cells. Only single Hassall' body is shown in this section. an Gieson stain. 200

Fig 2B. Higher magnification of Fig A. I the centre is an atrophic Hassall' body van Gieson stain 800

C_4, and bet $_{1C}$-globulin measured according to (8) were present in the same range as in normal sera

Immunoglobul ns Total serum proteins amounted to 6.5 %. Paper electrophoresis showed no peak in the gamma region, but quantitative measurement (Spinco Analytrol) ga e a value of 0 6 % for gammaglobulins. Paper electrophoresis, however gives un reliable too-high values, especially in the case of low gammaglobulin concentrations. *Gammaglobul ns were demonstrabl immunologically in concentration / 95 mg 100 ml* The analyses were carried out by the anti



Fig. 3A. Section from a lymph node with hyperplastic follicles and large necrosis. Only scattered lymphocytes are present in the medulla. van Gieson st. ×100.

Fig. 3B. Higher magnification of the section shown in Fig. 3 A. The hyperplastic reticulo-endothelium is shown. In the picture to the left there is necrosis with only a small number of granulocytes and lymphocytes at the periphery. van Gieson st. ×200.

globulin inhibition method and the gel diffusion precipitin technique as described in [16], and by an agglutination inhibition test in which the gammaglobulin in the specimen inhibits the reaction between anti-gammaglobulin and formalin- and tannic-acid-treated sheep red cells coated with human gammaglobulin [13].

It could be shown by immuno-electrophoresis (Scheidegger micromethod) a de-

scribed in (21) carried out with rabbit anti human plasmaprotein serum and specific rabbit anti-beta$_{2A}$ and anti-beta$_{2M}$ sera that the 7-S gamma globulin was much lower than normal, and that *no beta$_{2M}$ globul n and only traces of beta$_{2A}$ globul n could be demonstrated*

Investigation of relatives. It was found with the aid of paper lectrophoresis and immuno-electrophoresis that the father had a low serum concentration of beta$_{2M}$ globulin. The sister mother an aunt, an uncle and a cousin to the mother showed no immunoglobulin anomalies.

Treatment and course The boy s general condition remained fairly good despite the gradual development f recurrent pyrexia (37.8°-40°C) He was treated at the Hospital for Infectious Diseases with streptomycin and penicillin, and t the Department of Paediatrics with penicillin in million-unit doses, in both nstances with temporary effect. On January 19th 1962 he suddenly deteriorated despite all therapy and died 15 days after admission t hospital.

Necropsy

Thymus (Fig 2A and 2B) The thymus was markedly hypoplastic (1 7 g) Histologica examination showed very pronounced lymphoid hypoplasia with only very small, scattered groups of lymphocytes persisting The picture was dominated by numerous hyperplastic reticulo-epithelial cells. Only a few isolated, atrophic Hassall's bodies were present.

Lymph nodes and other lymphoid tissue (Fig. 3A and 3B). A few lymph nodes the size of pea and with angular gelatinous appearance were present in the region of the abdominal aorta. No other lymph nodes could be detected with the naked eye. The tonsils were very small. Microscopical ex amination of the enlarged nodes disclosed marked changes. The cortex contained only small, atrophic lymph follicles. Scattered lymphocytes were present in the medulla. On the other hand, there was hyperplasia of the reticuloendothelial component The cortex especially showed multiple areas of necrosis, with narrow peripheral zones of lymphocytes and occasional granulocytes. The parenchyma was oedematous.

The lymph follicles of the intestinal mucosa showed only isolated lymphocytes, and the reticuloendothelium was possibly hyper plastic. No plasma cells could be seen

Spleen (Fig 4) This organ was slightly enlarged (23 g) Microscopically the Mal pighian bodies showed moderat atrophy and were surrounded by narrow zones of haemorrhage The reticuloendothelium was moderately hyperplastic but part from this the medulla contained few cells, with only occasional lymphocytes and granulocytes in the sinusoids. There were no areas of necrosis.

Liver (Fig 5). The liver was enlarged (350 g) Large, yellowish-whit foci were visible t the naked eye On microscopical examination they were seen to consist of large partially confluent areas f necrosis surrounded by a few lymphocytes and gra nulocytes. In and about these areas of nec rosis there were numerous gram-negative rodshaped bacteria. There were no inclusion bodies.

Adrenals. The size was normal. The cortex contained multiple small areas of necrosis with no inflammatory cellular reaction. Gram-negative rods were also present in these areas of necrosis.

Bone marrow (*iliac crest*) No pathological changes in erythropoiesis were demonstrable N plasma cells were seen, but myelopoiesis was otherwise pparently normal

Brai Small, scattered patches of peri vascular lymphocytic infiltration were pre sent in the basal ganglia, but no areas of necrosis were seen.

Lungs. Small areas of atelectasis and haemorrhage were noted, but there were no signs of pneumonia.

Bacteriological and virological i vestigation of the postmortem material. Bacteriological culture of blood, lung liver spleen, kidney and gut yielded luxuriant growth of *Pseudomonas aeruginosa* in all samples. The gut also contained *B coli.*

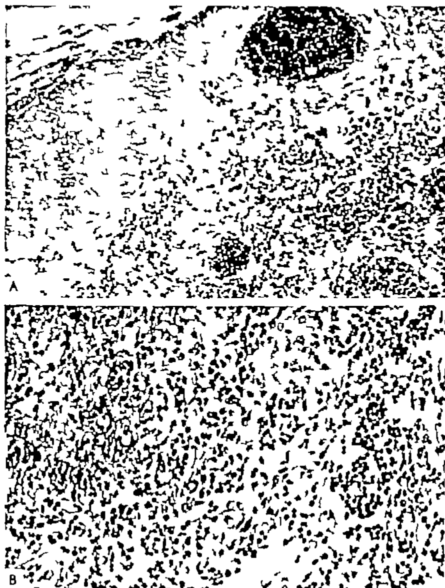


Fig 3 A. Section from lymph node with hypoplastic f llicles and a large necrosis. Only scattered lymphocytes are present in the medulla van Gieson stain 100

Fig 3 B Higher magnification of the section shown in Fig. 3 A. The hyperplastic reticulo-endothelium is shown. In the picture to the left there is necrosis with only a small number of granulocytes and lymphocytes at its periphery van Gieson stain 200

globulin-inhibition method and the gel-diffusion precipitin technique as described in [15], and by an agglutination-inhibition test in which the gammaglobulin in the specimen inhibits the reaction between anti gammaglobulin and formalin and tannic-acid treated sheep red cells coated with human gammaglobulin [13].

It could be shown by immuno-electrophoresis (Scheidegger micromethod as de-

Resumé of the necropsy findings

The essential changes were marked lymphoid hypoplasia, most pronounced in the thymus and lymph nodes, and reticulo-epithelial and reticulo-endothelial hyperplasia in the thymus and other lymphoid organs. The Hassall bodies were small and scarce. A noteworthy finding was the absence of plasma cells in the bone marrow and gut where such cells are normally present. The cause of death would seem to have been bacterial sepsis combined with generalized virosis. The multiple necrotic foci in the liver lymph nodes, and adrenals were most probably caused by infection, since large numbers of bacteria were demonstrated in connexion with them. The total or nearly total absence of inflammatory cellular reaction about these foci was striking

Discussion

The diagnosis of agammaglobulinaemia is based primarily on the immunochemical and serological laboratory findings, with the additional support of the clinical and morbid-anatomical picture. The term agammaglobulinaemia implies the total absence of gammaglobulins. With the aid of the sensitive methods of analysis now available however it is noteworthy that this is practically never found, although they may be greatly reduced. Strictly speaking then, ours was a case of hypogammaglobulinaemia. The term agammaglobulinaemia is an established one however and there is reason for retaining it

Most cases of agammaglobulinaemia with a typical clinical and/or pathological picture have shown gammaglobulin values of under 100 mg/100 ml as determined by specific immunochemical methods [1 10 11 14]. In certain cases however the values have exceeded this limit, and some have been described in which the gammaglobulin level was normal or raised but in which the patient nevertheless showed clinical signs of antibody-deficiency syndrome. In the latter type of case other of the antibody-carrying globulin fractions (beta$_{2M}$ and beta$_{2A}$) were often abnormally low

In our case the serum content of gamma globulins as determined by specific immuno-chemical techniques was 95 mg/100 ml. Even with regard to the age of the patient, this is very low though not extremely low Similarly beta$_{2A}$ and beta$_{2M}$ globulins determined by immuno electrophoresis showed a concentration that must be regarded as definitely pathological, even having regard to the patient's age.

Serological investigations revealed a very low content of isohaemagglutinins, and in addition, with one exception (Coxsackie B$_4$ neutralization test at titre 1/160) negative reactions to a series of different tests, including tests for antibodies against adenovirus type 2, although this virus was demonstrated in a number of organs post mortem

The absence of plasma cells from blood and bone marrow and the striking pathological changes well in keeping with agammaglobulinaemia in the thymus and lymphatic system provide further evidence in favour of the diagnosis in our case

Cases of agammaglobulinaemia are classified into three groups, on the basis of aetiology clinical course etc., namely 1) Congenital, 2) Transitory and 3) Acquired.

The cquired form usually appears after

the age of 1 year in connexion with some other primary disease. The debut at 3 months in our case and the fact that no other primary disease was present constitute evidence against this form of agammaglobulinaemia. It is impossible to distinguish congenital and transitory agammaglobulinaemia in a patient of 5 months of age by means of immunochemical and serological methods. The diagnosis is given by the subsequent course and repeated investigations when the patient with the transitory form of the condition again shows normal values, whereas in cases of congenital agammaglobulinaemia the immunochemical findings never return to normal. The presence of plasma cells in lymphatic tissue is said to indicate the transitory form [11].

In brief then the findings in our case are against acquired agammaglobulinaemia, transitory agammaglobulinaemia cannot be excluded, but the data tally best with a diagnosis of congenital agammaglobulinaemia.

To date 9 cases of fatal generalized BCG infection have been published [9 16, 17]. In none of these were complete serological investigation or determination of immunoglobulins carried out. It is stated with regard to the most recent of them [9] that using an unspecified electrophoretic technique gammaglobulin estimation gave widely varying results (1 0 and 0.2 g/100 ml) These findings would fit in with a defect in the gammaglobulin fraction. Electrophoresis, and especially paper electrophoresis give unreliable often too-high values, however particularly when the gammaglobulin levels are low

In addition to the immuno-chemical and serological findings, certain other observations may be of importance in establishing or excluding the diagnosis of agammaglobulinaemia. It is worthy of note in retrospect, that the brother of the patient now described, who died of generalized BCG infection several years ago, showed certain signs in keeping with agammaglobulinaemia

The post mortem findings of the two brothers in the thymus and lymph nodes tally closely in certain important respects (cf. the plates published in an earlier paper [5]) In both cases there was marked lymphoid hypoplasia or atrophy in both thymus and lymph nodes and in the thymus only isolated small, atrophic Hassall's bodies were present. These findings are all consistent with a diagnosis of agammaglobulinaemia. Further numerous epithelioid cells were found in thymus and lymph nodes in the BCG case but no tubercles with necrosis and Langhans's giant cells could be demonstrated despite the presence of great numbers of tubercle bacilli. In the case now described there was hyperplasia of the reticuloepithelium and reticuloendothelium in both thymus and lymph nodes. Reticuloendothelial proliferation in lymphoid organs has been described previously in agammaglobulinaemia [1]. In the light of these observations it is therefore conceivable that the epithelioid cell reaction described in the BCG case might also represent some change in the reticuloepithelial and reticuloendothelial cells. The pathological changes in BCG tuberculosis are to be subject of further study [7]

Yet another morphological sign of agammaglobulin defect in the brother with BCG sepsis is constituted by the pulmonary changes. These were at the time found

difficult to interpret. It has since transpired that they are entirely in agreement with Pneumocystis carinii pneumonia [6] Children with agammaglobulinaemia are clearly predisposed to infection with Pneumocystis carinii. It is important to bear in mind the possibility of agammaglobulinaemia especially in sporadic cases of this illness [3]. When Pneumocystis carinii infection attacks previously healthy children the characteristic pulmonary changes include a marked plasmacell reaction, but in children with agammaglobulinaemia this is complet ly or almost completely absent. In the BCG case plasma cells were present but in small numbers. This observation corroborates th suspicion of a defect in immunoglobulin production.

If to this is added the observation that the younger brother had agammaglobulinaemia, probably of congenital nature the evidence that the fatal BCG infection may have affected a child with agammaglobulinaemia is further strengthened.

The effect of BCG vaccination in agammaglobulinaemia is not fully understood. The tuberculin sensitivity evoked by the BCG vaccination is regarded as belonging to the delayed allergy group and as being cellbound. A number of children with agammaglobulinaemia have been immunized with BCG Many of these have shown an apparently normal reaction, but the proportion of them showing a transitory weak, or no reaction has been higher than normal. Barandun has reported the cases of 2 children who remained tuberculin negative after 3 and 6 BCG vaccinations respectively [1]. In the few known cases of agammaglobulinaemia in which natural infection with tuberculosis has occurred it has been impossible to say with certainty whether the course of the disease differed from normal.

Even though it is impossible to reach absolute certainty there are thus good reasons for believing that the fatal BCG sepsis that occurred in the family in question affected a boy who had agammaglobulinaemia. Both states are very rare It would therefore be remarkable if their occurrence together were purely coincidental. Rather would it seem that agammaglobulinaemia under special, unfortunate circumstances may give rise to a grave generalized BCG reaction. It is possible that the age of the patient is important in this connexion, BCG vaccination in newborn in this situation involving risks that are not present in older children. It is therefore wise to exercise caution in the BCG vaccination of infants known to have agammaglobulinaemia notably during the first year of life Unfortunately it is impossible to exclude cases of agammaglobulinaemia when BCG vaccination is performed routinely during the neonatal period, except when some sibling or other relation has shown signs of the condition. Further it is imperat ve that any new cases of generalized BCG infection be minutely investigated with regard to the possibility of agammaglobulinaemia.

Summary

The case is described of a boy who died at the age of 5 months showing the characteristic picture of congenital agammaglobulinaemia This child s brother died t the age of 8 months of generalized BCG infection, his sister is 6 years old and

healthy Several findings indicate that the brother also had congenital agamma globulinaemia. The importance of thorough immunological investigation of cases of generalized BCG infection is stressed.

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CASE REPORT

Jejunal Atresia with Intestinal Aplasia

Strangulation of the Intestine in the Extraembryonic Coelom of the Belly Stalk

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Atresia of the small intestine is a relatively uncommon malformation. It is characterized by a complete obliteration of the bowel lumen. The distended bowel proximal to the obstruction is in the majority of cases separated from the distal collapsed intestine by the atretic area which may consist of a solid cord.

The etiology of congenital intestinal atresias have been discussed since the beginning of the 19th century Several early reports suggested that prenatal mechanical obstructions such as volvulus, intussusception and strangulation might be the cause This idea was never in the past generally accepted. The theory based on observations by Tandler [12] and Forssner [3] that the human duodenum passes through a solid stage during embryonic life and that the development might arrest there has been thought a more likely etiology During recent years further observations have again directed attention to prenatal mechanical factors. It now has been possible to confirm these clinical observations with modern investigations such as micro-angiography and experimental work on living fetuses of animals.

In this paper a case of jejunal atresia will be presented in which there also was aplasia of the entire intestine from the atretic segment to the distal transverse colon This segment of colon ended blindly in the umbilicus. The atretic jejunum was also connected to umbilicus but by a solid cord of tissue. The case will be shown to support the theory that strangulation of the intestine in the extraembryonic coelom of the belly stalk can lead to intestinal atresias.

Case Report

B., a premature male, weighed 1810 g at birth and was the third child of a 40-year-old woman. Pregnancy and delivery were normal. Some hours after the birth the baby began to vomit greenish bile-colored intestinal contents. Through gastric tube 40 ml of bile-colored fluid were aspirated.

Plain films of the abdomen at 35 hours of age showed a greatly distended duodenum and jejunum with no gas in the bowel distal to the jejunum. A barium enema showed a "microcolon" and contrast passed only to the distal transverse colon (Fig. 1)

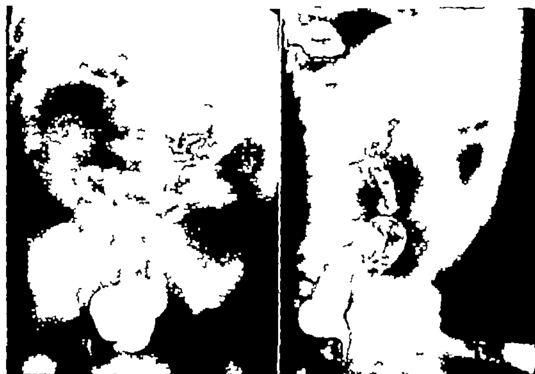


Fig. 1 X ray of the colon. Barium passed only t the distal transverse colon. The duodenum and jejunum are greatly distended

Surgery was performed through a right rect is muscle-retracting incision. There were no signs of peritonitis. The jejunum, which ended blindly was 15 cm long, uniformly distended and had a considerably thickened wall. The atretic ending was connected with the umbilicus by a very thin cord 5 cm long (Fig. *). The remainder f the small intestine was not present The distal colon ended blindly in the umbilical ring. The colon proximal to the distal transverse colon was also missing An end to-end jejuno transverse colon anastomosis was performed. The baby died on the fourth post-operat ve day with sclerema neonatorum.

Pathology (Björn I emark, M.D): In the two specimens from the j junum and the colon, taken from the region f the anastomosis, all layers of the i testinal wall were present with the exception of the *lamina muscularis mucosae* which was only present in small areas.

Discussion

In this case of jejunal atresia the pathogenesis may be fully explained by an incarceration of the bowel in the extra embryonic coelom of the belly stalk

During the sixth week of fetal life the midgut is situated in the umbilical cord, extra-abdominally During the 10th week there is a gradual 'reposition into the abdomen (Fig 3) If that "reposition" does not occur the hernial aperture closes around the intestine causing incarceration and gangrene The necrotic intestine is resorbed. This helps explain why part of the bowel is missing and why both the proximal atretic and the distal intestine terminate in the umbilicus.

During the last part of the 10th century



Fig. 2. Operative field. Transverse colon (C) terminates blindly in the umbilicus (U). The greatly distended atretic jejunum (J) is connected with umbilicus by thin solid cord (on the figure running partially behind the splenic flexure).

reports of cases of intestinal atresia were published and mechanical factors such as strangulation, volvulus, incarceration and intussusception were mentioned as possible etiological causes [1]. In 1902 Tandler [1] showed that human duodenum between the 5th and 8th weeks of development passes through a solid stage. This embryologic observation was confirmed by Forssner [3], Kreuter [5] and Johnson [4]. Until recently the cause of atresia has been considered to be an arrest of development

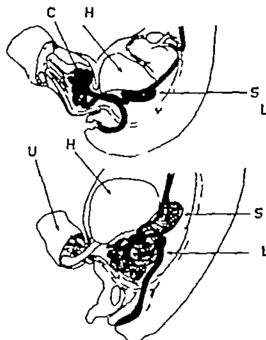


Fig. 3. Position of the intestine in the umbilical cord of 23 mm long embryo and return of the intestine into the abdominal cavity (embryo 45 mm). *C* cecum; *H* hepar; *S*, stomach; *L*, descending colon; *U* umbilicus. (The drawing modified after an illustration by Brodel in Cullen *Embryology and Diseases of the Umbilicus*. Courtesy W B. Saunders Co., Philadelphia, 1916)

at this stage Chiefly during recent years this theory of imperfect recanalization has been refuted by Morton [8] Nixon [9] Louw & Barnard [6] and Parkkulainen [11] Modern techniques in animal experimentation have made it possible to reproduce intestinal atresia by intrauterine ligation of mesenteric vessels with resulting atresia of the corresponding intestine [6] However in four consecutive infants with atresia Nixon [9] was able to show by angiography on the resected specimens that the vascular supply to the mesentery of the atretic segment was intact. Despite these cases Nixon and Louw *et al* agree that pathogenesis of some intestinal atresias is related to interference in the vascular supply of the completely formed fetal bowel. Nixon has supported this hypothesis with a case of atresia that developed following prenatal intussusception and pointed out the fact that atresia in this case developed in bowel that was already completely formed. He also has suggested that atresia could be caused by strangulation of the extraembryonic intestine by the umbilical ring In support of that he described a child that was born with an omphalocele and with a spontaneous double-barrelled ileostomy at the umbilicus and concluded that this was the result of incarceration of the "physiological umbilical hernia"

It is puzzling that in Nixon's case a double-barrelled ileostomy but not an atresia developed. The explanation may be that incarceration occurred late in pregnancy in an infant with an omphalocele and that peristalsis and the meconium content of the bowel were able to keep the lumen open In our case however incarceration probably occurred before the beginning of the 4th month. At this time there is little bowel content as no meconium is formed and there is little swallowing of amniotic fluid. There is also virtually no peristalsis. These factors allow the lumen of the intestine to close and result in atresia

Our case thus lends support to the theory that strangulation by the umbilical ring of the intestine while it still lies in the extraembryonic coelom may lead to atresia and aplasia of the intestine The fact that both the atretic segment and colon were connected to the umbilicus clearly supports this theory

Summary

A case of jejunal atresia is presented in which there was aplasia of the entire intestine from the atretic segment to the distal transverse colon. The distal colon ended blindly in the umbilicus. The atretic jejunum was also connected to umbilicus but by a solid cord of tissue

The case is described as support to the theory that strangulation of the intestine in the extraembryonic coelom of the belly stalk can lead to intestinal atresias.

During the 6th week of fetal life the midgut is situated in the umbilical cord, extra-abdominally During the 10th week there is a gradual reduction into the abdomen. If that reduction does not occur the hernial aperture around the intestine closes, causing incarceration and gangrene. The necrotic intestine is resorbed. This helps to explain why part of the bowel is missing and both the proximal atretic intestine and the distal intestine terminate in the umbilicus.

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CASE REPORT

Prader Willi Syndrome in Boy of Ten with Prediabetes

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In 1956 Prader Labhart, Willi & Fanconi [7 8] described a syndrome mainly characterized by mental deficiency obesity abnormally short stature muscular hypotonia and undescended testes. The patients in whom they observed this syndrome had all been floppy infants In 1961 Prader & Willi [9] brought the number of cases up to 14 and reported that the patients over 12 years of age often had diabetes mellitus. Shortly afterwards, Laurance [6] recorded six more cases. The same year Dunn & Miller [4] pointed out that the patient Dunn *et al* [3] had described in an article on congenital hypotonia must have had the Prader Willi syndrome at the same time they added three more cases

The syndrome is easy to distinguish clinically from other conditions. It is probably not as rare as would appear from the number of cases reported As yet nothing definite i known about its pathogenesis.

We shall now describe a new case After wards we shall analyze the clinical observations made to date to see if we can discover a plausible cause for the syndrome

Case Report

The patient a boy of 10 had been in homes for the mentally deficient ever since he was an infant

Family history

The parents were not related. The mother was a war refugee the last of four siblings in a Rumanian family; a large amount f infor mation was available on this family but it contained nothing of interest in the present case. The father was born out of wedlock to Swedish parents and had five healthy half siblings. The patient had a half-sister three years older a sister one year older and a brother three years younger these three children were healthy and of normal intelligence.

Past history

The boy was normally delivered after an uncomplicated pregnancy but he was born four weeks later than expected. When he was delivered he was lethargic and slightly cyanotic. His birth weight was 2850 g

The physician who examined him when he was born noted muscular hypotonia with flabby dangling limbs, poor development of the lower jaw and no cartilage in the external ears. The child also sucked poorly It was concluded that he suffered from a congenital disease of the muscles

The boy was examined again at the age of 1 At that time he was 7 cm tall, and weighed 9 6 kg and his head was 45 cm in circumference He had three teeth in his upper jaw and four in his lower H could not keep his head steady and he could not it unsupported and sat badly even when he was supported When he was laid face downward he kept himself up on his forearms, not on his hands. He showed a desire t get



Fig. 1 At the age of 10 the patient still has difficulty in standing and in walking without support.



Fig. 2. The physical peculiarities are particularly noticeable in the back view Not the bnormal accumulation of fat on the buttocks and the poorly developed lower legs and feet.

into contact with the people round him; he babbled and tried t grasp things. H still had severe muscular hypotonia, but his achilles tendon and knee-jerk reflexes were normal. H had no scrotum, and no testes could be palpated.

H continued t develop at an extremely slow pace both in motor and mental functions. Not until he was 1½ did he lift his head when he was laid face downward, and not until he was 2 did he lift his head when he was put on his back. When he was he was ble *to st y in a sitting position without* being supported but he could still not raise himself int sitting position. When he was between 4 and 5 he began t crawl. When he was 7 he could stand if he was supported. At 8 he learned t rise t a standing position himself. At 9 he could walk if he was helped, and at 9½ he learned t take one or two steps on his own. He had good emotional contact with his warders the whole time though he sometimes got int a bad temper He made extremely little progress mentally but at no time did he revert t a lower level. H never learned to speak; he began making monotonous grunting noises at the time a child would normally begin to talk, and he never advanced any further

Present condition (age of 10)

Examination when he was 10 revealed the following: He was 116 cm tall and weighed 24 kg. H had a body like that of an old *woman, with a large accumulation f fat on* his buttocks and thighs, the lower part f his bdomen, and his breasts (Fig. 1 and 2) His legs from half way down his thighs to his feet were thin and underdeveloped. His feet were extremely small and narrow only 16 cm long; they were hyperflexible and held in an infantile valgus position. His hands were also thin and slender but not so bnormally small as his feet H had normal palm prints. His head was slightly conical in shape; it was





Fig. 3. Fig. 4.

Fig. 3 and 4. The external genitals. Not the rudimentary scrotum.

31 cm in circumference and narrowed off toward the top, ending in a rounded dome. He had a peculiarly shaped face: he had a broad and projecting lower jaw and a broad nose but his palpebral fissures were normal. His ears were extremely soft and had poorly developed cartilage; each had a different shape but neither of them was particularly deformed.

He wa more retarded mentally than he was emotionally. H was affectionat and got along well with his warders, but he got very upset at times. He beha ed like a child of one or younger; according to the Vineland scale he behaved like a one-year-old in most respects; but he could not speak at all. He got 1 ½ points in the Vineland test t the age of 9 years and 9 months, which is equi valent to an S Q of 7.5.

Palatum ogivale was present. The teeth were badly decayed. A dentist who was consulted could not find any anomalies of development. The germs were present for all the permanent teeth. The mucous membranes were normally moist.

The superficial lymph nodes and thyroid were normal. Ordinary physical examination of the heart revealed nothing of particular interest nor did the ECG. The blood pressure was 95/55. The lungs appeared to be normal from physical and roentgen examination. The abdomen was covered with flabby skin, no abnormal masses were palpated there; neither the liver nor the spleen were palpable.

The external genitals are seen in Fig. 3 and 4. Either the testes were completely undescended or the boy had none. Instead of a scrotum he had an area of scrotal like skin about 2 cm in diameter with a suggestion of a raphe down the middle. His penis was small. His skin was dry but otherwise normal.

Neurologic examination revealed no paralysis, but all the muscles f the body were lacking in tone and strength, especially in the legs and feet. The muscular t one was

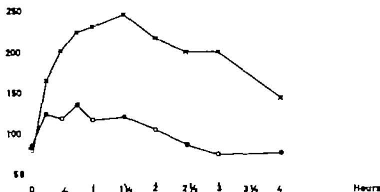


Fig 5 The glucose tolerance curves before and after the intra-muscular injection of 50 mg of hydrocortisone 3½ and 2 hours before the glucose was given.

poorly developed everywhere, but particularly distally in the limbs. The boy walked with a waddling and unsteady gait of the ataxic type: the gait was not of the cerebellar variety however and no tremor was present. On the other hand, he had difficulty in maintaining a sitting or standing position. There was no nystagmus and no hyperkinesia. The arm reflexes and the knee jerks were normal, the achilles tendon reflexes were exaggerated. The plantar response was normal on both sides.

It was impossible to measure the visual acuity but there did not seem to be any severe defect in vision. The eyes were normally placed and moved normally and the media and eyegrounds were normal.

The EEG taken when the boy was awake was of immature type but otherwise normal. The electromyogram showed intense insertion activity in the short toe extensors, but no other sign of muscular disease or peripheral neurogenic injury. The conduction velocity was normal in the right peroneal nerve between the knee and ankle.

Roentgenograms of the skull were normal. Films of the facial bones showed great protrusion of the jaws. Films of the spine showed that the arches of the 3rd, 4th and 5th lumbar vertebrae were fused together. Soft tissue films of the limbs showed poorly developed muscles and wide bands of fat between the bundles in the thighs. The bony nuclei were normal for the boy's age. On encephalography the whole ventricular system filled well, but no dislocation or deformity could be seen. The right lateral ventricle was 22 mm wide as against 72 mm for the inner diameter of the skull on the same side and the left was 25 mm wide as against 70 mm for the inner diameter of the skull on that side: these measurements indicated that the left lateral ventricle was abnormally wide. The fourth ventricle was unusually wide. The basal cisterns were of normal shape. Small amounts of air were seen on the convexity in grooves of normal width.

Analysis of the blood showed hemoglobin 1_.7 g/100 ml; red blood cells 4 400,000; white blood cells 8300, including 5000 polynuclear

TABLE 1 *Urinary output of 17 ketosteroids and 17-hydroxycorticosteroids before and after provocation with Metopiron and ACTH*

The provocation tests were done at an interval of two weeks.

Steroids in mg per 24 hours	Before provocation (Values on three non-consecutive days)	After 11-beta hydroxylase inhibitor (3 g by mouth) 0–24 hours after	24–48 hours after	After ACTH (20 I.U intramuscularly) 0–24 hours after	24–48 hours after
17 Ketosteroids	0.6, 0.7 1 4	0.3	1 0	1.0	1.3
17 Hydroxycorticosteroid (Norymberski)	0.3, 0.8, 3.0	1.3	..4	4.1	4..

cells; thrombocytes 281 000· reticulocytes 3.6%; MCV 88; MCHC 33%; colour index 0 93 micro-S.R. 1 mm/ 1 hour; nonprotein nitrogen 40 mg/100 ml, serum creatinine 0.7 mg/100 ml, total bilirubin 0 4 mg/100 ml directly measured bilirubin 0 1 mg/100 ml; thymol 1 1 units; serum calcium 5.0 mEq ; serum phosphorus 4.09 mg/100 ml; serum protein 7 8 g/100 ml serum electrophoresis normal. Analysis of the plasma lipids showed cholesterol 219 mg phospholipids 234 mg glycerophospholipids 164 mg sphingomyelins 84 mg triglycerides 48 mg all per 100 ml of plasma.

The urine was examined on several occasions, but no albumin or glucose was ever found and the sediment was always normal. Two-dimensional paper chromatography of a 24-hour output showed a normal amino acid pattern; 10.5 mg of amino nitrogen were excreted per 100 mg of creatinine. There was no phenylpyruvic acid in the urine.

Examination of the cerebrospinal fluid showed normal pressure; negative Pandy and Nonne reactions; total protein 4 mg/ 100 ml; negative reaction t the mastic test; two mononuclear cells per 3.3 mm³; negative Wasserman reaction.

The ordinary glucose tolerance test gave a normal curve with a peak value 1 138 mg after 45 minutes. When the test was repeated after the intramuscular injection of cortisone (50 mg of hydrocortisone 8½ and bours before the glucose was given), the curve was both elevated and delayed (Fig. 5)

Hormone analysis revealed: tri iodothyronine uptake 18% and PBI 8 4 gamma/100 ml; less than ..5 units of gonadotropins per liter urine. The urinary output 1 17 ketosteroids and 17 hydroxycorticosteroids per 24 hours was low both before and after provocation with Metopiron and ACTH and gave the values shown in Table 1

Analysis of the chromosomes in 2. cell from cultures f the skin and 11 from cultures of the blood showed a normal chromosome complement with 22 pairs f autosomes and n XY gonosomal constitution.

Summary of case The most noteworthy observations were severe mental deficiency short stature a peculiar type of obesity undescended testes and only a rudimentary scrotum, abnormally small hands and feet and muscular hypotonia. The patient had been a floppy infant.

Injection of cortisone caused an elevated and delayed glucose tolerance curve. According to Conn & Fajans [ ] this is a strong indication of a prediabetic state implying that although he still maintains normal carbohydrate metabolism the ability of his beta cell to respond to

further stimulation is considerably limited"

In our opinion, the clinical picture in this case was characteristic of the syndrome described by Prader *et al*

Discussion

The clinical features are compatible with a genetically determined disease The syndrome has never been found in more than one sibling of a family however nor has any other form of familial occurrence been observed. No case has been reported in which th parents were related, it is positive that they were not related in one case of Dunn *et al* [3], in all six of Laurance s cases [6] and in the present case Thus, so far nothing has been observed that definitely points to hereditary transmission of the syndrome

The chromosomes were normal in 11 out of the 12 cases in which they were examined. In one case of Prader & Willi [9], in all six of Laurance [6], in three of Dunn & Miller [4] and in our case In the twelfth case described by Dunn *et al.* [3], cells from two cultures of skin and muscle contained an extra small acrocentric chromosome This difference indicates that even though the cases are alike in clinical respects, som of them may be of different origin than others Apart from this one instance of chromosomal aberration, however no distinct evidence of any other prenatal causes for the syndrome has been seen so far

Nineteen of the altogether 25 patients reported have been boys and six girls. This is a significant deviation from the usual 50 50 distribution ($0.25 > P > 0.01$) The lack of a normal scrotum is a very noticeable abnormality in a boy however and so one is probably more apt to notice the syndrome in a boy than in a girl.

It is interesting from the point of view of pathogenesis to examine the main clinical components of the syndrome It is convenient to divide them into four groups, though it is impossible to avoid over lapping The four groups are peculiarities in body shape endocrine disorders neuromuscular defects and mental deficiency

Peculiarities in body configuration The most characteristic physical abnormalities are a special type of adiposity hypogenitalism affecting the scrotum in particular extremely short stature small hands or feet, or both Our patient was not especially obese but he had disproportionate amount of fat on his buttocks, thighs and lower part of his abdomen, much more than seen in certain types of brain lesion e.g residual conditions after hydrocephalus. The degree of hypogenitalism seems to vary from case to case the scrotum was amazingly underdeveloped in our case however and also in the case described by Dunn *et al* [3]. The patients are also very short. It is true that mentally deficient children are often short but children with the Prader Willi syndrome grow disproportionately as well, our patient s lower legs did not grow at the same pace as the rest of his body and his hands and feet were as small and narrow as those of a doll. Acromicria appears to be one of the few characteristic signs of the syndrome

Besides these abnormalities of diagnostic value there are usually one or more minor congenital deformities, such as lack of cartilage in the external ears, high palate clinodactyly partial syndactyly small defects in the bones and delayed development of the osseous nuclei Dunn & Miller

[4] also observed irregularities in the teeth especially in the enamel. The number of these defects seems to differ greatly from case to case

Endocrine disorders The syndrome is always characterized by subnormal endocrine function. Prader & Willi found evidence of primary hypogonadism several of their male patients excreting an abnormally large amount of gonadotropins. In our case there was evidence of subnormal activity in the adrenal cortex. The low output of 17 hydroxycorticosteroids in the urine after stressing with ACTH and Metopiron pointed either to hypothalamo-hypophysial disorder or to primary dysfunction in the adrenal cortex or both There was no other clinical evidence of primary malfunctioning of the adrenal cortex however and so the more probable alternative seems to be a hypothalamo-hypophysial disorder Two of Prader & Willi's five children over 12 had diabetes of the old age type and one had prediabetes. One of Laurance's [6] patients had a decidedly prediabetic glucose tolerance curve after provocation with prednisone Our patient who had no sugar in his urine gave a diabetic response to the cortisone-glucose tolerance test indicating that the beta cells in his pancreas did not have enough reserve capacity This laboratory evidence of insufficient endocrine functioning is borne out by the old woman shape of the patients bodies.

Neuromuscular defects The number and type of neuromuscular defects differ from case to case apart from the amyotonia congenita which all the patients show in infancy It has been demonstrated recently that amyotonia congenita may occur in diseases of greatly varying origin Dunn *et al* [3] and other authors have gone into this subject in detail. Children with this form of amyotonia often manifest their hypotonia and weak muscles before they are born by making only feeble fetal movements. After they are delivered, they have difficulty in breathing and are asphyctic they cry feebly and suck poorly As they grow older they make poor motor progress again mostly because of hypotonia.

Neither electroencephalography electromyography nor studies of the nerve conduction velocity seem to be of any help in diagnosis or in determining the pathogenesis Nor is neuro-radiologic examination of much help Pneumoencephalographic examination gave nothing in the cases of Prader & Willi, in our case it showed only wide cerebral ventricles. Histologic studies have only been done by Dunn *et al* they observed unusually narrow fibers in specimens of muscle but no other change of note

Mental deficiency Prader & Willi found that the patients were usually imbeciles, having I Q.s between 40 and 50. The patient described by Dunn *et al.*, however had an I.Q of approximately 80 at the age of 2½ Our patient deviated in the opposite direction, having an I Q of no more than 20

The patients seem to develop much better emotionally than they do intellectually Prader & Willi described their patients as good natured children with the same silly contented expression on their faces from morning to night Our patient exhibited a fairly wide variety of emotion, in spite of his severe retardation

In short the syndrome is characterized by subnormal reserve capacity in the endocrine neuromuscular and mental

functions. None of the clinical data indicate that the abnormalities are apt to progress on the contrary the patients seem to grow better in some respects. They give the impression of suffering from an inhibition in development caused by prenatal factors. The most plausible common denominator for them all is a developmental anomaly in the central nervous system but it is impossible to speculate further with the information we now have.

As mentioned, Dunn *et al* [3] observed a chromosomal aberration in one case but the other 11 cases in which the chromosomes were examined showed a normal karyotype. It would seem from this that the syndrome is not the single entity it has appeared to be after all it has only been recognized for a short time and there is still much to learn about it. Support for this assumption is contained in a recent article by Bühler *et al* [1]; they described a boy with a syndrome very like the Prader Willi variety in which they found a chromosome number of 45 and a consistent karyotype pattern with only four large acrocentric chromosomes of group 13–15 and an additional metacentric chromosome interpreted as the result of a translocation of the two missing chromosomes of group 13–15

It may be that there will prove to be several different varieties of the Prader Willi syndrome If so, it will no longer seem peculiar that some cases show a chromosomal aberration and others not

Summary

The authors describe a boy of 10 showing mental deficiency short stature a form of obesity typical of endocrine dysfunction undescended testes and only a rudimentary scrotum minor congenital deformities acromicria and muscular hypotonia Laboratory analysis revealed a low urinary excretion of 17-keto-steroids and 17 hydroxycorticosteroids, and a diabetic response to a cortisone-glucose tolerance test, pointing to prediabetes. The boy showed amyotonia congenita in infancy and so his condition tallies well with the syndrome described by Prader & Willi. The etiology and pathogenesis of this syndrome are discussed in the light of the clinical data assembled to date.

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PROGRESS IN PEDIATRICS

The Secular Change in Growth and Development

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Increase in Body Size

A striking acceleration in the growth of children is taking place in many parts of the world. The change has been observed not only in the United States and Western Europe but also in Japan, Argentina, Esthonia, Slovenia and elsewhere. The difference is considerable. During the past century the average height of adults in Western Europe and USA has increased by 10 cm [39].

The trend toward more rapid growth started in Europe more than 100 years ago. There was little change in the size of Norwegian adults between 1760 and 1830 [24]. Then between 1830 and 1875 adult height increased by about 0.3 cm per decade and by about 0.6 cm per decade between 1875 and 1935. Changes in height had already been noted in Italian recruits between 1791 and 1841 [11].

The more rapid growth of children begins during fetal life. The weight at birth has been rising since the middle of the last century. The weight of Swedish newborns increased by several hundred grams in the 85-year period between 1850–60 and 1035–45 [28]. Larger newborns are to be expected as adults grow larger, since there is a correlation between size of the newborn and the size of the parents [3].

After World War II a temporary decline in weight and length at birth took place in Germany, France, Holland, Italy, Japan and Russia. Curiously, a similar drop also occurred in Switzerland which was a non-combatant. No significant change in birth weight and length was observed in Sweden between 1918 and 1945 [1].

Growth during the first year of life is also more rapid now than formerly. Length at one year has increased from 0.3 cm to 75.3 cm in the 70-year period between 1850–1909 and 1920–1941 [33]. In the 1890's New York infants at 1 year were about 72.5 cm long and weighed about 9.0 kg (Table 1) [22]. Forty years later [4] the average length of infants at one year whose care was specially supervised, had increased by almost 3.5 cm and the weight by 1.5 kg in boys, and by 1.3 cm and 0.0 kg in girls.

Table 1 also includes the measurements of infants at one year reported by Falkner [15] in 1962. These infants do not differ significantly from those who received good medical care in the 1930's. Although the

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TABLE 1 *Comparison of the heights and weights of infants at 1 year in 1897 1936 and 1962*

Author	Year	Height (inches)		Weight (pounds)	
		Males	Females	Males	Females
Holt [22]	1897	29.0	28.7	20.5	19.8
Bakwin & Bakwin [4]	1936	30.6	29.9	23.8	21.8
Falkner [15]	1962	30.2	29.4	23.3	21.7

weight of well looked after infants has not increased since the 1930 s, it is probable that babies in general are larger now since more are receiving proper medical supervision.

Broman Dahlberg & Lichtenstein [10] pointed out well marked accelerations in the growth of school children over the years Between 1883 and 1938–1939 height increased by about 9 % and weight by about 20 %. They found no correlation between the duration of the nursing period and the height and weight after 1 year Nor could a correlation be established between the age of the mother and the dimensions of the child

Boyne & co-workers [9] deduced that in the group which they studied between the years 1911 and 1931 all the changes in growth had taken place during the pre school years However in the next decade 1931–1941 the acceleration did not begin until the early school years. A similar conclusion was reached by Melbin [31] in a study of Lapp children. He found that the improved diet and general environment at modern boarding schools failed to affect the children s development He inferred that the main effect of the environment on children s growth and development takes place in the pre-school years (under 7 years)

In the study by Greulich [17] comparing the growth of native born and American born Japanese children well marked differences in height and weight were already present at 6 years of age when the earliest measurements were made

In Fig 1 the growth of children in the general population, measured in the 1930 s, is compared with children measured recently [18]. Growth of the boys is about the same in the two periods up to 9 to 10 years after which the recently measured boys surpass those of the 1930 s in both height and weight. Girls measured recently are larger than those in the 1930 s from infancy The discrepancy begins to widen at about 8 years.

Evidently an increase in the size of children in the general population is continuing

In the 1930 s we also measured children from private schools in New York City These children, who presumably had received the best available care were not only taller and heavier than the public school boys at that time (Fig. 2) they were also taller and heavier than the children recently reported by Reed & Stuart [35] and by Falkner [15]

All the children used in our study were white and with rare exceptions, were born in the United States The children were

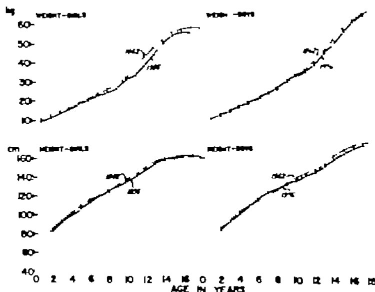


Fig 1. Comparison of weights and heights of children in the general population measured in 1936 and recently

divided by ethnic origin into four groups, North European Middle European, Mediterranean and Jewish (mainly eastern European)

The only consistent difference found was that the children of Mediterranean background (mainly Sicilian) were somewhat shorter than the others.

The private school population was made up almost entirely of North European and

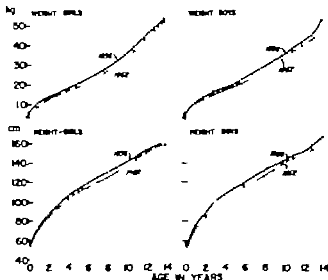


Fig 2. Comparison of heights and weights of private school children, measured in 1936, and children from the general population measured recently

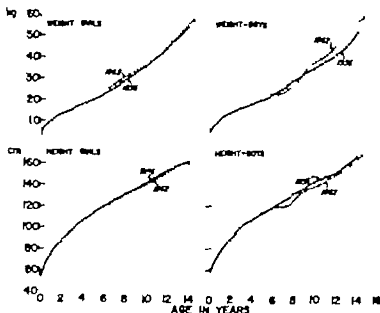


Fig 3 Comparison of heights and weights of private school children measured in 1930 and in 19

Jewish children No differences in growth were found between these two groups.

In 196_ we again measured children, ranging in age from 6 to 14 years from the same private schools used in the 1930 s (Fig 3) No change in the selection of students has taken place at these schools during the past 30 years. The boys, measured in 1962, weighed more after 9 years of age than those measured in the 1930 s otherwise the heights and weights of the girls and the heights of the boys were if anything smaller than in the 1930 s. Apparently by the 1930 s the children who had received optimal care had reached their maximum size Lenz [_3] points out that in Camerer s material which was collected during the last quarter of the 19th century among well to-do German families the average size of 19 year-old males was 170 7 cm the same as that of German students today

We may however expect that the size of the general population will continue t increase until they reach the size of th private school population.

Reasons for the More Rapid Growth

The reason or reasons for the more rapi growth are unclear The factors affectin the rate of growth have been criticall reviewed by Tanner [30 p 94]

Illness Though repeated infections hav been found to be associated with retarde growth [20], it is not clear whether th frequent infections produce the growt retardation or whether the smaller childre are more susceptible to illness. Tanne concludes that "most of the information o the effects of minor childhood illness tend to contradict the perhaps generally bu vaguely held notion that illness interfere with normal development"

During a major illness growth may b retarded but recovery is accompanied b accelerated growth. Nephrod is known t

retard growth but it is doubtful that the retardation is permanent.

Psychological factors Emotional tension influences appetite increasing it in some instances, depressing it in others.

Emotional deprivation in infants reduces appetite and slows growth even when food intake is adequate [5]. If the infant is removed to a favorable environment a prompt improvement in appetite and acceleration in growth ensue The effect of long-standing emotional deprivation on growth, in the face of an adequate dietary intake has not been studied.

Widdowson [4] was able to demonstrate that adverse psychologic conditions may retard growth during childhood. She studied the children in two orphanages in Germany during the difficult post war years At one point the diets in one of the orphanages was supplemented, in the other it remained unchanged. Contrary to expectation, the children in the orphanage where the diet was supplemented grew less rapidly than the children in the orphanage where the diet remained unchanged.

The reason for this paradoxical result appeared to be that at the time the dietary improvement was made a new nurse was placed in charge of the orphanage where the diet was supplemented. This woman previously in charge of the other orphanage was an unusually severe disciplinarian who demanded implicit obedience Moreover she often chose meal time to administer unjustified rebukes. This so upset the children that they often left their food uneaten.

It is likely that strict disciplinary attitudes, prevalent during the 19th century and the early decades of this century had an adverse effect on children s appetite A measure of the baleful influence of the rigid discipline during those years was the high suicide rate among children in certain parts of Germany France Russia and elsewhere

Tanner quotes a considerable amount of evidence showing that school stress may influence physical growth adversely

Miscellaneous factors That the number of children in a family is associated with growth changes is an old observation [7] In less well-off economic groups the children in large families are on the average appreciably smaller than those in smaller families and menarche takes place later This is not nearly as marked in the higher socio-economic groups. The difference in size between members of small and large families persists into adult life In part at least, the retardation is socio-economic that is, among the less well-off classes, the more children to care for the less general care and food for each one Tanner [20 p. 143] concludes that "the family size effect differs in no way from the general socio-economic class effects and is susceptible to similar explanations

Exercise No clear cut relation between growth and exercise has been demonstrated.

Nutrition Undernutrition during childhood slows growth and delays the appearance of the adolescent spurt. This is unquestionably a retarding factor in a large part of the world where the food supply is short. Temporary periods of growth retardation have also been observed in European countries during times of famine subsequent to war But this can hardly account for the secular trend in Western Europe and U.S where prolonged, severe food shortages have not occurred during the past century

At least three changes have taken place in the diet of American infants since the turn of the century: the widespread substitution of cow's milk for human milk in infant feeding, the routine supplementation of infants' diets with vitamin D and the early introduction of solid foods.

Cow's and human milk differ from each other in innumerable respects. More important, the reaction of infants to the two milks is different. Observations made a number of years ago [14] showed that breast fed babies grew differently from the artificially fed. The breast fed babies weighed more during the early months of life but at 4 to 5 months they were surpassed by the artificially fed. Similar results were obtained in Sweden [32] in 1950 using modern feeding methods. May [30] has pointed out that the growth of breast fed babies from birth to 6 months has remained unchanged in various studies reported since 1893.

Infants receiving cow's milk retain more calcium, phosphorus and nitrogen than do those on the breast. The blood lipids, which reflect the body lipids, also differ with the two types of milk [43]. The plasma level of vitamin C is lower and large amounts of C are necessary to raise the plasma level to that ordinarily found in the breast fed baby. Other differences are the greater frequency of tetany (both newborn and D deficiency) and eczema in babies receiving cow's milk, the lesser resistance to respiratory infection, an altered reaction in the blood sugar level to lactose ingestion, a difference in the intestinal flora. Most of the infants in our study group were partially or completely breast fed for from 3 to 6 months. All were given vitamin D supplement and orange juice from the age of 3 weeks and solid food at about 3 months.

An acceleration in growth in height of infants has been observed with supplements of 340–400 units of D a day and with exposure to sunlight [37]. On the other hand, it was not possible to detect any differences in the growth of prematures receiving various preparations of D in doses of 100 to 800 units daily [16].

The widespread practice of incorporating vitamin D into the diets of infants in temperate climates may have helped to promote growth. It is not known whether the accelerated growth due to added vitamin D persists after the first year.

For the last 30 or 40 years it has been customary in the United States to introduce solids into the diets of infants at about 3 months rather than at 6 months or later which was the earlier practice. More recently the time of feeding solids has been advanced so that many babies receive solids at a few days of age on leaving the obstetrical hospital. The very early feeding of solids does not seem to have affected the growth of infants since no change has been observed during the last 30 years.

Dietary intake varies in subtle and not always readily comprehensible ways. For example in U.S. the consumption of fluid milk has been falling since 1950 presumably due to the "cholesterol scare." The use of fluid cream has been diminishing since the early 1930's and in 1961 was only 68% of that in 1947–49. The consumption of butter has also been declining since the early 1930's and here factors such as the poor quality of American butter, the availability of adequate substitutes, the anti-cholesterol propaganda are considerations.

Per capita consumption of cheese and frozen dairy foods (including ice-cream) has increased.

The consumption of milk varies widely from country to country Though fluid milk is drunk fairly freely in the United States, the consumption of milk and milk products is less than 50% that of Ireland Finland and New Zealand.

How such variations in the consumption of milk and milk products affect human growth is not known

Socio-economic status There is speculation as to the reasons for the socio-economic difference in growth In a Swedish study [2] slower growth in poorer families was found to be related not so much to income as to social status. The type of work in which the father was engaged proved to be a more important influence on the growth of children than his income A good correlation could be demonstrated between the type of employment of the father on the one hand and the height and weight of the children on the other while no correlation was found between the father's income and the size of the children.

A likely explanation is that parents doing a higher type of work, are more intelligent than those doing inferior work and are consequently better able to select a proper diet for their children. This is borne out by our experience In the 1930's [4] it was possible to improve the growth of infants from an economically deprived group and being it up to that of a middle income group simply by giving advice about the diet and general management.

Accelerated Maturation

A second developmental change which has been taking place during the past century is the earlier appearance of puberty and adolescence The characteristic growth spurt and menarche are appearing earlier and growth is completed at a younger age In Norway [8 16 41] the age of menarche has declined from 17.. years in 1850 to 13.5 years in 1950 a difference of 3 7 years. A similar decline has been observed in Finland, Sweden, England, Germany and U.S.A. [39]. The average age of girls at menarche in U.S is between 12.5 and 13 years, varying somewhat with socio-economic status and region.

At the same time as the menarche is appearing earlier the menopause is taking place later

A number of environmental factors are known to influence the time and character of the adolescent process More rapid growth and better nutrition are accompanied by earlier maturation. Thus obese children mature earlier than others.

Socio-economic status is an important factor Girls from lower status families menstruate later than those from more favored families In New York City menarche occurs about 9 months earlier among well-off negroes than in negroes in the southern states where the economic situation is much poorer [34]

There is a widespread belief that menarche appears earlier in underdeveloped peoples and in tropical climates The data do not bear this out. The mean age of menarche in upper class Nigerian girls is actually higher than in England [13] and is just about the same as in Alaskan girls [28] In New York City no difference was found between white and negro girls of similar socio-economic status [34] A curious observation, made by two different

observers, is that menarche occurs later in darker haired children [8 27]

Not only are the adolescent spurt in growth and menarche appearing earlier maturity is reached at a younger age Whereas maximal height was probably not reached in men until about 26 years some 50 years ago the age now in high socio-economic groups in United States and Western Europe is about 18 to 19 years in boys and two years earlier in girls.

Emotional development Marked changes in emotional reactivity are characteristic of adolescence Basically these changes are determined biologically but they are profoundly influenced by cultural factors. Accompanying the marked growth in the size and activity of the sex organs, interest in the opposite sex is greatly increased. Increased activity of the adrenal hormones is believed to be related to increased aggressiveness and the drive for social dominance Since the emotional development is so closely tied to the hormonal development it seems reasonable to assume that emotional development during adolescence is now taking place earlier than formerly

Mental development It is difficult to determine whether a spurt in mental development takes place during adolescence owing primarily to the character of mental tests and how they have been set up. Eichorn & Bayley [12] on the basis of studies in a small group of children studied from birth, present data which strongly support the view that there is a spurt in growth of the head circumference during adolescence

In their group the growth spurt took place at 11 to 1 years in girls and three years later in boys corresponding to the years of maximum growth in height The increase in head circumference was greater than could be accounted for by the increased thickness of the skull and soft tissues. The inference is, therefore that a spurt in growth of the brain takes place during adolescence In view of the observation of Eichorn & Bayley the former view that a spurt in mental development does not occur during adolescence must be reassessed.

Body Composition

There is ample evidence that the body composition can be altered by changing the dietary constituents The differences in the body composition of infants receiving human and cow's milk have already been described.

More recent studies have dealt principally with the response to alterations in the character of the fat in the diet. The composition of body fat (heart liver testis, cerebrum) has been profoundly altered in chickens by feeding diets containing different fats [20] Changes in body lipids have been produced by feeding rabbits diets supplemented with cholesterol and certain combinations of fatty acids [38].

In man the composition of the adipose tissues has been changed by feeding diets rich in various fats for long periods of time [19] The fatty acid content of breast milk ha also been altered by dietary manipulations without affecting milk volume or milk fat output [43]

It i clear then that body composition can be altered by dietary manipulation. This opens up basic problems. Are some of the differences which we consider to be racial actually due to differences in the

foods eaten? Diets vary widely in different parts of the world. Are the changes in the brain lipids reflected in differences in mental functioning?

Discussion

What is the significance of these profound changes in growth and development? We can readily see how the increased size of children and adults will make necessary many adjustments. Growth standards for children must continually be altered if they are to be useful. Standard clothing sizes for children have already been revised upward. Seating arrangements in public places—schools, theatres, cinemas, trains, aeroplanes—will have to be adjusted for the greater comfort of a larger kind of man. Beds and bathtubs must be made bigger. More food will be necessary to support the larger individual.

Of more immediate importance to the physician is the earlier maturation of young people. Earlier maturation has prolonged the difficult period of adjustment between the time when drives for self-expression, social dominance, sex experience, economic independence appear and the time when these drives and desires can be satisfied. The interval has been further extended at the other end by the increased number of students who continue in high school and college and who consequently remain economically dependent on their parents. The trend towards specialization has still more lengthened the training period.

Must we revise downward our estimates of the proper time to terminate compulsory schooling, to choose a career, to enter military service, to marry, perhaps to vote? Should we revise our attitude toward the age of responsibility? It is customary to exempt the young from responsibility for certain criminal acts and also for managing their own affairs, for example marriage, inheritance and the like. The law protects children from many special dangers such as the use of alcohol, imprisonment, premature marriage. Earlier maturation suggests that we loosen the reins earlier in matters of compulsory schooling, child labor laws, marriage and so on.

It may be argued that earlier maturation is not the only consideration in judging the age of responsibility. Childhood and adolescence are years of *preparation for adult life.* Earlier maturation means that the period of preparation has been shortened and that maturity is now being reached before the youth has had an opportunity to accumulate experience in the way his later maturing predecessors had.

Against this argument is the fact that opportunities for accumulating experience are much greater now than ever before, what with the trend toward urban life where the tempo is greatly accelerated over that in rural communities. Radio and television, too, are great forces in widening experience.

Summary

For the past 100 years or more children and adults have been growing taller and heavier. The increased size is probably due to changes in the character of the diet. It seems reasonable to assume that the end is in sight, that the ultimate size reached will be that already achieved some years ago by well-looked-after individuals as represented by the private school population.

Adolescence is beginning earlier and maturity is being reached earlier. Earlier maturation poses many problems in management especially as psychologic conflicts between parent and child appear earlier and last longer.

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Acute Head Injuries in Children

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(*Supplement 152*)

The material of this investigation comprises all the 305 children with acute head injuries in the age group 0–14 who were admitted to and treated in the Surgical Clinic at the Kronprinsessan Lovisa Children's Hospital in Stockholm in one calendar year. The material confirms that acute head injuries in children as a rule are of a mild character and give rise only to rapidly disappearing somatic symptoms, with a low frequency of complications. A few days observation in hospital is recommended. The duration of confinement to bed shall be as short as possible and adjusted to the subjective and objective symptoms.

In all cases a careful and detailed traumatological anamnesis was recorded when the child was admitted. Its somatic and mental status were recorded on standardized forms. The material for the first half year was subjected in addition to an intensive psychiatric scrutiny. This scrutiny included, on the one hand, the recording of a comprehensive anamnesis with parents and teachers furnishing the information and, on the other, a certain amount of psychotherapy for the parents. Six months after discharge from hospital the patients were followed up as regards the occurrence of neurological and psychiatric symptoms.

It was clear from the investigation that *inter alia* the patients constituted a special selection. A remarkably large number came from homes with mentally ill or social parents, a remarkably large number had parents with anxious and grossly exaggerated fears of the sequelae of head injuries, a remarkably large number had already had head injuries previously or had been involved in other accidents, and a remarkably large number had already manifested pronounced symptoms of mental illness before the head injury.

At the follow-up examination only a small percentage of the patients were stated to have had neurological sequelae of the head injury. Mental sequelae were reported in about 10 % of the cases and invariably in children who came from an insecure home environment or who had already shown symptoms of mental illness previously. No child who came from a normal home environment and had previously been mentally healthy had had mental symptoms after the head injury. The children of the parents who did not receive psychotherapeutic treatment had a significantly higher frequency of psychiatric symptoms after the head injury than the children of the parents who received this treatment.

The results are discussed and practical steps for obviating mental sequelae in children with head injuries are indicated.

Acta Pædiatrica 53 91 94, J n. 1964

PROCEEDINGS OF PEDIATRIC SOCIETIES

Swedish Pediatric Society

Meeting Sept 21 1963

Ralph Holman, John Lind and Lars Söderhjelm Unsaturated F tty Acids in Serum from Breast Fed Infants and Cow Milk Fed Infants

Using the alkali isomerization technique the content of dienoic, trienoic and tetraenoic acids in serum has been determined in newborn infants and in infant fed human milk and cow milk. In breast fed infants there is an increase in dienoic and tetraenoic acids with time whereas in serum from cow milk fed infants, the content f these unsaturated fatty acids remains low

Åke Gyllenswärd and Stig Malmström Exchange Transfusions at the Hospital in Boden

Boden is the center of Norrbotten county which covers one-fourth of Sweden, with about 260,000 inhabitants. At the hospital, there are about 4500 deliveries a year In 1955–1962, 134 exchange transfusions were performed, 93 in Rh-immunizations. Eighty per cent refers to the last four years with about 30 exchange transfusions a year Indications and technique are adapted to the general norms in Sweden. Delivery is induced in selected cases up t 4 weeks before expected time. The prenatal death-rat among the Rh-immunized during this period was bout 10 Mortality and complications have been studied in detail, and follow-up examination performed. In the total series, the death-rat was 2 per transfusion and 3 per child. In the Rh-cases 4 and 5 % respectively; no death occurred in immediat connection with the exchange transfusion and there ha been no deaths during the last 2 years. No significant complications have occurred during the transfusions since we stopped giving calcium through the catheter in 1960, which sometimes resulted in alarming bradycardias.

No cases of kernicterus have occurred either in the exchanged cases or in the Rh immunizations without exchange transfusions. The two cases of kernicterus included bad hyperbilirubinemia without immunization and were not sent to us until the cerebral symptoms had started. The results tally closely with others and thus the exchange transfusion service in this hospital seems satisfactory especially when regarded from a geographical point of view

Per Kählin and Sylvia Garme Hedman Experiences of Exchange Transfusions in Umeå

In a district in northern Sweden covering about 65,000 sq.km. some 4500 deliveries per year occur in 14 hospitals. All cases in which exchange transfusions may be necessary are admitted to the General Hospital at Umeå. In the years 1956 t 1963 93 cases were treated with 131 exchange transfusions and re-examined. Indications for exchange transfusions are: positive direct Coombs test a hemoglobin of 14 g or less, and an indirect bilirubin of 3.5 mg or more in cord blood. Asphyxia, prematurity or erythroblastosis in siblings indicat ex change even if the last two criteria are not completely fulfilled. Exchange is also per formed if the increase in serum bilirubin exceeds 0.5 mg /hour during the first 36 hours. If the serum bilirubin exceeds 20 mg for otherwise healthy mature infants, 23 mg % for premature or asphyxiated infant

before the age of 7 days, exchange also has been performed.

Case A was the 6th child of a supposedly Rh(+) woman with five healthy children. Hyperbilirubinemia beginning on the first day increased to 3* mg on the 3rd day when convulsions occurred. Three exchange transfusions were performed. The child is now mentally retarded, but without athetosis. The mother was found to be o immunized. *Child* B was a post mature child of a primipara. Icterus started on the 3rd day with opisthotonus and athetoid movements. Admitted at the age of 7 days with a serum bilirubin of 37 mg. The child, now 2½ years old is mentally and physically retarded. *Case C* was the 2nd child of an Rh immunized diabetic mother delivered by cesarean section in the 33th week. As the child was severely asphyxiated he was not sent for exchange transfusion until the third day. Died on the 4th day from respiratory distress. *Case D* was the fourth child of an Rh-immunized mother. Exchange transfusion without complications. The infant was mongoloid with an atrial septal defect and died at the age of 6 weeks of cardiac failure. *Case E* was a firstborn mature infant with a bilirubin of 22 mg on the second day. The mother was 4 immunized. As the icterus did not decrease in 1 days, an exchange transfusion was performed. Towards the end of the transfusion the child died from cardiac arrest. *Case F* had a bilirubinemia of 40 mg when admitted on the 8th day and died of kernicterus at the age of 2 months.

In our series of treated cases, the mortality is similar to other groups reported. The total number of Rh immunized pregnancies in the district is not known for this period. It is possible that some still births were not registered as erythroblastoses. Fifty-six per cent of the entire series are boys. Among the 16 crippled or dead infants, 12 were boys. Treatment has been successful in most cases. Complications are seen when patients are sent for exchange transfusion rather late after neurological signs are manifest. Better results might be expected after instruction of all categories of hospital staff on the significance of early jaundice in newborn infants.

P.-G. Bergfors: Sulphonamide Treatment Once Again

Penicillins are preferred perhaps too often to sulphonamides in the treatment of infections. Regarding both therapeutic safety and frequency of side effects, modern sulpha compounds are by no means inferior to penicillins. The cost of sulpha treatment is about one-third that of penicillin treatment. Recent research upon the pharmacokinetics of sulphonamides has demonstrated great discrepancies between different derivatives. It is necessary to use more individual dosage schemes than has hitherto been practised. The initial dose must be sufficiently high to permit a safe plasma concentration of active sulpha from the first day of treatment. Unfortunately most recommendations by pharmaceutical manufacturers seem to be erroneous on this point. Also the maintenance doses now generally in use are too low for many sulphonamides. For some of the older ones, however, they seem to be too high. A new sulpha derivative 3 methoxy-sulphanilamidopyrazin (Kelfizin[1]) has some outstanding qualities which makes it a real improvement in this field. Its elimination is considerably slower than any other sulpha derivative in use. The binding to plasma protein is very small in comparison with other long-duration sulphonamides. These and other properties of Kelfizin make it theoretically possible to use maintenance doses of only 50–100 mg once daily i.e., about one-tenth of other modern sulpha compounds. Thanks to this low dose symptoms of intolerance will probably be still more uncommon. Our preliminary clinical studies on the effect of these low doses of Kelfizin[1] in cases of urinary infections are promising. In contrast to other long-duration sulphonamides, Kelfizin[1] probably can be used even in cases of bacterial meningitis, as the passage to the spinal fluid is good with levels of 25–40% of the serum level.

Meeting Oct 11 1963

Bertil Hall Mongolism in Newborn Infants

With the object of evaluating different diagnostic criteria and thereby the possibility of diagnosing mongolism at birth, the author has for one year July 1961–June 196.., investigated all newborn infants in Southern Sweden suspected of mongolism with regard to clinical symptoms and chromosome pattern. During this period, 43 instances of suspected mongolism were referred for investigation. All were investigated according t a special formula. Of the 43 cases, 38 had the typical mongoloid hromosome pattern, trisomi 21 while the remainder were chromosomally normal. The number of confinements in this area is about 25,000 annually The mongol frequency is therefore bout 1/650 confinements, a frequency that corresponds well with the accepted value Two of the f e cases with normal chromosomes showed signs of mental retardation at the age of one year In a third instance mental retardation was suspected, but the infant died before this could be established with certainty Based on these observations, the uthor selected 10 cardinal clinical signs of mongolism in newborn infants. Primarily signs that could be assessed or easily defined were chosen. These cardinal signs included. oblique palpebral fissures, iris spots, four finger crease bnormal middle phalanx of 5th finger Ilio index <70° negative Moro reflex, protruding tongue circumference of head less than 32.5 cm, bnormal ears, and hyperflexibility

In this material mongoloid infants had 4–10 cardinal signs but most f them had 7–8 One of the chromosomally normal infants has 5 cardinal signs, including mongoloid type pelvis. These cases would also have been classified as mongolism, if one had chosen more subjective traditional signs as basis of comparison. Thus, it is not possible t recognize by these clinical signs all chromosomally normal infants, who give the impression of mongolism. Of the remaining chromosomally normal infants, none had more than 3 cardinal signs. On the basis of these observations, one should be able to diagnose mongolism clinically in newborn infants with reasonable certainty if 6 cardinal signs occur If fewer signs are positive a hromosome investigation should be carried out The chromosomally normal infant with 5 cardinal signs showed signs of mental retardation at the age of one year From a practical clinical point f view he must be regarded as mongoloid, but when he deviates in some respects, he should be classified as a paramongoloid.

Marianne Ohlson, Göran Sterner and Sigvard Wolontis Antibodies Against Respiratory Viruses Among Healthy Children

Sera from two groups of healthy hildren 1–3 years old (66 children) and 9–1.. years old (74 children) were examined for complement fixing antibodies against respiratory viruses: adenovirus, parainfluenza 1–3, respiratory syncytial virus and influenza A and B. More than half of the school children had antibodies against adenovirus, parainfluenza 1–3, and respiratory syncytial virus; the small children, however had antibodies against these viruses t a much smaller extent. Antibodies against influenza A and B were found only sporadically in these two groups.

G Sterner Marianne Ohlson, S Wolontis, Margareta Gullmar and G de Hevesy Infections with Respiratory Viruses in Orphanage and Nursery

From October 1959–January 1960 stools and sera were collected from normal infant (3–12 months old) living in an orphanage and during F bruary–March 1963 from children (1–3 years old) with mental retardation living in nursery The stools were examined for cytopathic agents. Only adenoviruses were recovered. Nine infants excreted adenovirus types 1 5 or 7 Paired sera showed a rise (4 fold) in CF titre against adenovirus antigen in three children.

One of them (an infant) had a symptomless infection, the other two had acute respiratory illness. Sera from the orphanage and the nursery showed a CF antibody titre of 4 or higher in 60 % and 90 % respectively. Infections with adenoviruses seem to occur twice as often in children living in the orphanage and the nursery as in children (1–3 years old) living in their homes. The same proportion was valid even for infections caused by parainfluenza 1. One-third of the children in both groups had CF antibodies against parainfluenza 1 but none against parainfluenza 2. Only 1 infant showed antibodies against parainfluenza 3 compared with one third of the children in the nursery. Three infants had a significant rise in CF antibodies against parainfluenza 1. One of them also had a rise in CF antibodies against parainfluenza 3. All of these last mentioned infants had been ill with acute respiratory illnesses during the study. Half of the children living in the nursery but none of the infants living in the orphanage showed a titre of 4 or higher of the CF antibodies against respiratory syncytial virus. Three of the children had an infection with this virus during the study. An outbreak of respiratory illness among the children with mental retardation just before this study started, was probably caused by respiratory syncytial virus. None of the children had detectable CF antibodies against influenza A and B.

Fulger Lagercrantz, Stockholm

NEW BOOKS RECEIVED

Books received by the *Acta Paediatrica* are acknowledged under this heading. Selected books will be reviewed in subsequent issues as space permits.

H. Loeb: Contribution à l'Etude du Métabolisme Energétique de l'Enfant. Editions Arscia, S. A. Bruxelles, 1963. Price Frs. B 350

E. Rossi (ed.): Neue Probleme bei Infektionskrankheiten des Kindes. Pädiatrische Fortbildungskurse für die Praxis. Vol. 10. S. Karger AG Basel–New York. 1963. Price S. Frs. 24.

P. J. W. Miller, R. M. E. Seal and M. D. Taylor: Tuberculosis in Children. J. & A. Churchill, Ltd. London, 1963. Price £6 net

H. Opitz and F. Schmid (Editors). Handbuch der Kinderheilkunde. Vol. V. Infektionskrankheiten. Springer Verlag Berlin–Göttingen–Heidelberg, 1963. Price DM 288.

Frederic Speer (Editor). The Allergic Child. Hoeber Medical Division, Harper & Row Publishers. New York, Evanston, and London, 1963. Price $16.50.

G. Jentschura, E. Marquardt and E. M. Rudel. Behandlung und Versorgung bei Fehlbildungen und Amputationen der oberen Extremität. Georg Thieme Verlag Stuttgart. Price DM 22.50

Jan Lindsten. The Nature and Origin of X Chromosome Aberrations in Turner's Syndrome. Almqvist & Wiksell. Uppsala, 1963. Price Sw. Kr. 32:—

First Inter-American Conference on Congenital Defects, Los Angeles, California, January 22–24 1962. J. B. Lippincott Company. Philadelphia and Montreal 1963.

Seminar on the Prevention of Antenatal and Perinatal Cerebral Lesions. Centre International de l'Enfance Mai 1961. S. Karger Basel and New York, 1963.

F. Unterharnscheidt. Die gedeckten Schäden des Gehirns. Experimentelle Untersuchungen mit einmaliger, wiederholter und gehäufter stumpfer Gewalteinwirkung auf den Schädel. Springer Verlag. Berlin–Göttingen–Heidelberg. 1963. Price DM 48.—

Kinderpsychiatrie in der Praxis. Postgraduate Courses in Pediatrics, edited by E. Rossi. S. Karger. Basel and New York, 1963.

F. Gross (ed.) Protein Metabolism. Influence of Growth Hormone, Anabolic Steroids, and Nutrition in Health and Disease. An International Symposium. Leyden, June 25–29 1962. Springer Verlag Berlin–Göttingen–Heidelberg, 1963. Price DM 37.50.

K. Schreier (ed.) Die angeborenen Stoffwechselanomalien. Grundlagen, Klinik, Therapie. Georg Thieme Verlag. Stuttgart 1963. Price DM 69.—

J. Colin & C. Polonovski. Explorations Biologiques en Pédiatrie. 2e édition. L'Expansion Editeurs. Paris, 1963. Price F 174.

G. Müller. Der plötzliche Kindstod. Pathologische Anatomie und Dynamik. Georg Thieme Verlag. Stuttgart, 1963. Price DM 29.70

BOOK REVIEWS

H Opitz and F Schmid Handbuch der Kinderheilkunde Vol. V: Infektionskrankheiten

Springer Verlag Berlin–Göttingen–Heidelberg, 1963 Price DM 360

The editors say that the "Handbuch" will be published in nine volumes and will treat all subjects in the field of pediatrics and child health with the exception of pediatric surgery which is considered sufficiently covered in Lehrbuch der Chirurgie und Orthopädie des Kindesalters edited by A Oberniedermayr The two editors of the book reviewed here H. Opitz and F Schmid, the former a well experienced editor of medical journals and books, have received contributions from 440 collaborators in Europe and U.S.A. The aim of the editors is to give a representative and exhaustive presentation of current pediatrics, emphasizing practical viewpoints. They hope that the whole series of volumes will be published within the next two years. In two of the volumes the interest of pediatricians in practice and in clinical work will be the "Leitmotiv" in the disposition of the material and a comprehensive survey of diagnostics, treatment social welfare pediatric institutions and biostatistics will be given.

The present volume is number V Infektionskrankheiten and in this all infections common among children in temperate zones and the most important infectious diseases in the subtropics and the tropics are exhaustively treated. As an introduction more general points of view are given in two short chapters. The division of the various kind of infectious disease conventional and the size of each separate chapter seems to be adequate The largest section is given to tuberculosis (225 pages) and is divided in 10 subchapters with different authors. Otherwise there is only one author for each disease In all this volume contains 1,59 pages with 418 partly colored figures.

To judge from the present volume the editors have succeeded fairly well in their aim and the volume tallies satisfactorily with the standard of the same volume in the old Pfaundler-Schlossmann's Handbuch. For the old generation of pediatricians the Pfaundler-Schlossmann played an important role and most pediatric practitioners had access to these volumes. It was also translated into English and published in the United States It is a pity that the price of the volume under review (subscription for the nine all volumes will lower the price to DM 289) will make it almost impossible for pediatricians in soft-currency countries to buy it

Claude Polonovski and Jean Colin Explorations biologiques en pédiatrie Deuxième édition

Expansion scientifique française Paris, 196[illegible] Price 174 Fr

This volume of 1104 large-size pages contains three parts: one giving technical descriptions of procedures in clinical biochemistry, one dealing with tests of different functions of the human body and one describing how these tests are used in clinical investigation. In the last part are found descriptions of symptoms and signs of various syndromes together with diagnostic schema, and in the latter two parts some clinical-physiological investigations and blood group serology tests are described. It goes without saying that even in a volume of this size all topics cannot be completely discussed For example the descriptions of serum glucoproteins and abnormal haemoglobins are not comprehensive but such important clinical investigations as those of bilirubin, haematocrit and pH in blood are only briefly discussed and no micromethods are mentioned On the whole however the book contains a wealth of information and up-to-date references

Lars Wrangsjö Uppsala

G. Fanconi and A. Wallgren: Lehrbuch der Pädiatrie 7th revised edition

Schwabe & Co Verlag Basel-Stuttgart 1963.

It is with great expectations that one opens the 7th edition of Fanconi-Wallgren text book of pediatrics. This book is characterized by its appearance in a new revised edition every second year and by the exchange of retired old authors by young specialized col laborators, giving the chapters always a modern and fresh content One may how ever hope that the two outstanding editors in future, although retired, will continue to publish this textbook, which has well merited its present distribution all over the world in six different languages. The book is presented in a nice typographical form with many illustrations, tables, graphs and diagrams giving the reader a visual impression which facilitates the understanding and helps the memory

While the 1st edition f 1950 contained 864 pages, the 7th edition has grown to 1143 pages. It is perhaps somewhat too big for undergraduat students, but on the other hand it will serve as a valuable handbook not only for pediatricians but also for general practitioners.

In a short review it is not possible to enter into details, but one should like t stress that it would be desirable in the next edition to give a more exhaustive description of poiso- ing in childhood, which plays such an important role in everyday practice.

N pediatric department should miss this textbook, in which t is easy to find the information one wants, thanks to a general index that covers 83 pages.

Carl Friderichsen, Copenhagen

Howells, J G Family Psychiatry

Oliver & Boyd, Edinburg and London, 1963. Price 18 s.

Many are the project which have been advanced in the field of social and psy hia tric act vity for the prevention and cure of emotional disorders in children and adults.

The Department of Family Psychiatry in Ipswich under Doctor Howells presents, however a new and excellent approach which aims to help the family as whole by coordinating all the forces in the community The clinic not only collects information from different key persons and community agen cies but also gets the active cooperation of these instances in the treatment of the family Each member of the family no mat ter which one has applied for help, is dealt with individually according to need. In spit of this complicated procedure the result seems to be natural and organic so that each member as well as the family as a whole shows improvement This is in contrast t other methods, where after therapy with only one member of a family the others as well as the family as a whole may show a deterioration in their situation Doctor Howells program would seem like an ideal mental hygienic preventati Does he how ever really believe that even if we could prevent children during their development from coming in contact with unstable persons in key positions we might expect in a few generations a more emotionally stable popu lation? Emotional disorder is unfortunately not a homogeneous concept and the solution can scarcely be so simple Nevertheless the book is very stimulating well written, clearly thought out concisely expressed and so much better than we are accustomed to in this field. No individual who is interested in the "to be or not t be of the modern family can afford t miss reading this book.

Elsa-Brita Nordlund Stockholm

Tuberculosis in Children by Edith M Lincoln and Edward M. Sewell with foreword by Arvid Wallgren.

McGraw Hill Book Company Inc New York, 463. Price $1_.95.

In 1922 Edith Lincoln began a clinical and pathological study of tuberculosis in the Pediatric Department f Bellevue Hospital in New York City Between 1930 and 194 1342 children with primary pulmonary t ber

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BOOK REVIEWS

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Lars Wrangsjö Löppeda

noticed period of successive deterioration has preceded the onset of frank disease. It is reasonable to assume that the number of children with mild–moderat forms of protein-calorie malnutrition in a given commun ity grossly exceeds that of the severe cases. In preventive health work and from the public health point of view it is of great interest to find some simple criteria f defining the children belonging to the former group.

Dr G Arroyave discussed "Biochemical signs of mild–moderat forms of PCM" Several authors have found that a low protein intake will diminish the ratio urea N total N in the urine. Likewise the ratio urea N creatinine is reduced. Single morning urine specimens can be used for a kind of screening procedure The urea-N creatinine is not infallible, since poor nutrition may also reduce the creatinine excretion. Analyses f individual amino acids in the blood plasma indicat a definit change in PCM with low levels for the essential amino acids but usually normal or even raised levels for the non-essential ones. So far only severely ill children have been studied.

Dr J C. Waterlow reviewed our know ledge of "Metabolic disturbances in PCM" In spit of much work we are unfortunately far away from having any good and early laboratory signs of the mild–moderate forms. It may well be that we must concentrate on trying t find and measure quantitative deviations that are still within physiological limits. As far as functional tests are concerned, since there is usually a large physiological reserve, positive results are more likely to be found if the function is loaded or stressed This latter possibility was taken up by Professor R. F A. Dean in his lecture on Production and control of oedema" Surprisingly in studies with different supply of sodium and potassium it came out that under the given conditions (4 g protein and 100 cal/kg/day) an addition of 2 g sodium chloride right from the beginning of treat ment was therapeutically beneficial.

Professor W J Darby discussed "Adapta tion to suboptimal nutrition with respect to protein and calories" and Dr F W Lowen stein "The vicious circle mechanism in the production f PCM" In the discussion Dean stressed the fact that "there is very seldom only one factor responsible for the malnutri tion of the individual child. It is as though a few insults can be borne but when too many insults simultaneously arrive the child goes down hill

Dr J Cravioto presented Mexican observations of "The influence of PCM on psychological test behaviour" They indicat a definite correlation between, e.g., IQ score and degree f malnutrition. Admittedly such correlations may be complex in interpretation but they are a challenge to continued studies along the same lines.

The prevention of PCM by utilization of protein-rich foods was discussed in some detail by Dr B. M. Nicol. With respect t short term prognosis the following discussion remark by Dean is of interest "W have followed-up 200 children treated since 1954 and 20 of them are known to have died, confirming the rarity of recurrences. Recur rences in our experience occur almost exclusively in children who are the victims of unfortunate psychological circumstances. In the 20 children who died, the most common mode f death was to fall down dead without any warning."

Other valuable contributions dealing with PCM (by Professor E. J Bigwood, Professor and Mrs. D B. Jelliffe, Dr B. Friis-Hansen) are found in the volume. Furthermore also a short presentation (by Professors Ågren Mellander and Vahlquist) f the NIB project "Children Nutrition Unit in Ethiopia and information on "Methods for the Determination f Physical Capability by Professor G Strőm.

Bo Vahlquist Uppsala

ANNOUNCEMENT

The IVth Middle Eastern Mediterranean Pediatric Congress

This Congress will be held in Athens from September 27 to 30, 1964 and will be sponsored by the International Pediatric Association and the local Pediatric Societies. Information from Professor C. B. Choremis, President, Pediatric Clinic of Athens University and Dr S. G Hadjiklakis, Secretary 29 Stadion Street Athens 122, Greece.

The XIV Nordic Pediatric Congress

The congress, under the presidentship of Professor Rolf Zetterström, will be held in Stockholm June 21–24 1964. The principal subjects to be discussed are:

1. Neonatal jaundice
2. Care of premature infants.
3. Congenital malformations, including chromosomal aberrations.
4. Immunological anomalies.

For further information about the Congress, write to the General Secretary of the Congress, Associate Professor C. G. Bergstrand, Kronprinsessan Lovisas Barnsjukhus, Polhemsgatan 30 Stockholm K.

In direct connection with the congress a *Nordic Refresher Course in Pediatrics* is planned for June 25–26. The principal themes are: *Pediatric Neurology* and *Pediatric Endocrinology*. Request for information regarding this course should be addressed to Associat Professor Rutger Lagercrantz Department of Pediatrics, Karolinska sjukhuset Stockholm 60

Acta Pædiatrica 53 101–104 March 1964

From the Department of Biochemistry Baroda University Baroda, India

Studies on Human Lactation

Part IV

Lactic and Malic Dehydrogenases and Xanthine Oxidase in Breast Milk and their Variation with the Progress of Lactation and Dietary Vitamin Supplementation

by A. D DEODHAR, R. RAJALAKSHMI,[1] and C. V RAMAKRISHNAN

A number of enzymes have been detected in breast milk including lipase [1 5], acid and alkaline phosphatases [2, 4, 5 12, 13], and peroxidase [10]. Although lactic, malic glutamic and alcohol dehydrogenases have been reported in goat milk [8], their presence has not yet been demonstrated in breast milk. Preliminary investigations on the presence of these enzymes in breast milk led to the detection of lactic and malic dehydrogenases and confirmed the presence of xanthine oxidase. Studies were made on changes in these enzymes with the progress of lactation and with dietary vitamin supplementation

Experimental

The 90 subjects employed for these experiments were from amongst those used for investigations otherwise directed [3]. The collection f milk was done according t the procedure described elsewhere [3].

Chemicals

The chemicals used in the experiments were obtained from the following sources: diphospho-pyridine nucleotide from Sigma Chemicals, glacial acetic acid from E. Merck & Co., cysteine hydrochloride from E. Merck & Co., 1-lactat from La Roche, 1-malat from L. Light & Co., toluene and 2,3,5-triphenyl tetrazolium chloride from British Drug House Ltd.

Assay system for lactic and malic dehydrogenases

The assay system employed for the deter mination of dehydrogenase activity was a modification f that used by Sreenivasamurthy and Swaminathan [11], and consisted of potassium phosphat buffer pH 7.5, 100 μmoles; substrate 50 μmoles; cysteine hydrochloride (neutralised), 20 μmoles; $MgCl_2$, 5 μmoles; di phospho pyridine nucleotide 100 mcg; 2,3,5 tri-phenyl-tetrazolium chlor ide 50 μmoles; enzyme preparation, 0.5 ml; and distilled water to a final volume f 3 ml. The system was incubated at 37° for 2 hours. The reaction was stopped by the addition of 2 ml of acetic acid. The formazan formed was taken in 6 ml f toluene and the colour intensity measured at 470 mμ on Klett Summerson Photoelectric colorimeter The blank contained all the components except the substrate

A unit of enzyme activity is defined as the amount of enzyme which, under the condi tions of the assay causes a reduction of 1 mcg of 2,3,5-triphenyl tetrazolium chloride in 2 hours.

[1] C.S.I.R. Scientists- Pool Officer

TABLE 1 *Lactic dehydrogenase malic dehydrogenase and xanthine oxidase contents of different fractions of milk*

	Lactic dehydrogenase %	Malic dehydrogenase %	Xanthine oxidase %
Top fatty layer	5.0	23.0	40.0
Middle supernatant layer	5.0	77.0	60.0
Bottom residual layer	0.0	0.0	0.0

Assay of xanthine oxidase

The assay system was a modification of the one described by Zittle *et al.* [14]: 500 μmoles of phosphate buffer pH 7.5, and 1 μmole of hypoxanthine solution were taken in 1 5 15 mm test tube and the volume made up to 1.5 ml with water: dry nitrogen was bubbled through the reaction mixture for 5 minutes, 0.5 ml of milk added, and the nitrogen again bubbled through for another 5 minutes; 0.[illegible] ml of 0.05 M [illegible] 5-triphenyl tetrazolium chloride was then added and the final volume adjusted to 3.4 ml with water. The mixture was incubated at 0 for 10 minutes and the reaction stopped by the addition of 5 ml of glacial acetic acid. Four ml of toluene were then added and the mixture shaken vigorously to extract the formazan. The colour intensity of the toluene layer was read at 4 0 mμ. The blank contained boiled milk in lieu of fresh milk.

Preparation of milk sample for the determination of lactic dehydrogenase malic dehydrogenase and xanthine oxidase activities

On centrifugation at 4000 g and 0 for 30 minutes the milk sample was found to form three distinct layers: a top layer (fatty), a middle layer (supernatant), and a bottom layer (residual). The three layers were separated and the fatty layer suspended in 0.0 M phosphate buffer pH 7.5, the residual layer in 0.03 M phosphate buffer pH 7.5. All the three fractions were dialysed for 4 hours at

TABLE 2 *Lactic and malic dehydrogenases and xanthine oxidase in breast milk at different stages of lactation*

	Lactation period							
	Days				Months			
	1 7 (10)	7 14 (10)	15–21 (10	22 30 (10)	1 3 (10)	3–6 (10)	6–1 (10)	1 (10)
Lactic dehydrogenase (units/ml)	78 0 [illegible]0	74 0 ±1.[illegible]	44.0 ±1.4	40 0 ±1 4	[illegible]0 ±1 [illegible]	1.0 ±0 7	17.0 0 6	[illegible] [illegible]
Malic dehydrogenase (units/ml)	54 0 1 1	45.6 1 0	48.0 ±1.0	30 0 ±0.[illegible]	[illegible] 0 ±0 7	18 0 0 [illegible]	1[illegible] 0 [illegible]	[illegible] [illegible]
Xanthine oxidase (units/ml)	[illegible] 0 1 3	49.0 [illegible]	41.0 [illegible]	39 0 [illegible]1 7	34 0 [illegible]7	34.0 [illegible]	31 0 1 6	37 [illegible] [illegible]1 [illegible]

The values given are means of activity (units) per ml, with standard errors.
* The numbers in brackets indicate the number of subjects in each group.

TABLE 3 *Lactic and malic dehydrogenases and xanthine oxidase (units ml) in breast milk at different periods of supplementation*

(The control groups received no supplementation.)

	Prior to supple-mentation	Period of supplementation in months I	II	III	IV	V	VI	VII	VIII
Lactic dehydrogenase									
Experimental	33.0 ±1.8	32.0 ±1.5	29.0 ±1.0	26.0 ±0.9	22.0 ±1.0	18.0 ±1.0	14.0 ±0.9	14.0 ±1.0	14.0 ±0.9
Control	32.0 ±1.2	30.0 ±1.0	26.0 ±0.9	24.0 ±0.9	20.0 ±0.8	16.0 ±1.0	13.0 ±1.0	13.0 ±0.8	12.0 ±1.0
Malic dehydrogenase									
Experimental	24.0 ±1.0	23.0 ±0.8	22.0 ±0.9	21.0 ±0.9	21.0 ±0.8	19.0 ±1.0	15.0 ±1.8	14.0 ±0.8	14.0 ±0.8
Control	23.0 ±1.2	22.0 ±1.0	22.0 ±0.9	[illegible]2.0 ±0.8	21.0 ±0.7	18.0 ±0.8	15.0 ±1.0	13.0 ±0.8	13.0 ±0.7
Xanthine oxidase									
Experimental	37.0 ±1.5	36.0 ±1.3	37.0 ±1.4	37.0 ±1.3	35.0 ±1.3	36.0 ±1.3	35.0 ±1.3	34.0 ±1.7	35.0 ±1.8
Control	35.0 ±1.5	34.0 ±1.3	35.0 ±1.4	35.0 ±1.5	34.0 ±1.5	35.0 ±1.1	34.0 ±1.5	35.0 ±1.3	34.0 ±1.7

0 against 0.02 *M* phosphat buffer pH 7.5 and tested for xanthine oxidase, and lactic and malic dehydrogenase activities. The supernatant fraction was found to show maximum enzyme activity (T ble 1) and was therefore used in all subsequent experiments.

Results

The activities of the three enzymes at different stages of lactation are presented in Table _ and in groups with and without vitamin supplementation, in Table 3 It can be seen that with regard to all the three enzymes studied, the level of vitamin intake shows no relation to enzyme activity The ctivities of the two dehydrogenases are found to decrease steadily with the progress of lactation. That of xanthine oxidase however shows only initial decreases and remains at a steady level after the first month of lactation.

Discussion

The observation that the activities of the enzymes show no relation to vitamin intake raises the question as to whether the presence of these enzymes in milk is merely due to their association with cer tain microsomal particles secreted along with milk the escape of such cytoplasmic components having been suggested as a possibility by Morton [7] and Richardson [9]. In other words, the question arises as to whether the enzyme levels in milk have any relation to those in mammary tissues.

On the other hand the relatively higher activities of these enzymes soon after parturition and a gradual decline thereafter would appear to be consistent with the relatively higher rate of carbohydrate metabolism soon after parturition and a gradual decline thereafter in rat mammary tissue suggested by McLean s data [6].

Although the author infers a relatively stable level of oxidation of glucose-6-^{14}C as compared to the oxidation of glucose 1-^{14}C, the former increases from 0.8 in early pregnancy to 2.3 in early lactation and declines to a value of 0.6 at the termination of lactation these changes being statistically significant.

The significance of these observations is therefore far from clear and must remain a matter for speculation till further investigations are made

Summary

The presence of malic and lactic dehydrogenases was detected and that of xanthine oxidase confirmed. The activities of all the three enzymes were found to decrease with the progress of lactation and to be unaltered by dietary vitamin supplementation.

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The Effect of Parathyroid Extract on the Serum Concentrations of Calcium and Inorganic Phosphate in Active and Healing Rickets

by ROBERT STEENDIJK

Some years ago Harrison, Harrison & Park [3 5] showed that administration of parathyroid extract to hypocalcaemi vitamin D-deficient rats had no effect on the serum concentrations of calcium and inorganic phosphate (serum-Ca and serum P) When parathyroid extract was in jected 48 hours after administration of vitamin D serum-Ca rose and serum P fell.

Jonxis [6] reported similar findings in children. In the normal child parathyroid extract caused a rise of serum-Ca and a fall of serum-P whereas in the vitamin D-deficient child these concentrations were unaffected by parathyroid extract

These studies led to the conclusion that vitamin D has a permissive action on the effects of parathyroid hormone.

The purpose of the study presented here was to follow the effect of parathyroid extract on serum-Ca and serum-P during the healing of severe vit min D-deficient rickets with vitamin D in order to find out at which stage of the healing process a normal response to parathyroid extract could be elicited.

The subject was an 18-month-old boy with severe vitamin D-deficient rickets. H had been fed almost exclusively on milk and porridge and had never received vitamin D On admission to the hospital serum-Ca and serum P were 8.3 and 1 9 mg per 100 ml respectively; alkaline phosphatase was 35 Bodansky units; the concentration f serum-proteins was normal. A radiograph of the wrist is shown in Fig. 1A.

Administration of 1 000 U.S.P units of parathyroid extract (100 units twice daily for 5 consecutive days) failed to cause a consistent change in the levels of serum Ca and serum-P (Fig. 2) Following this, treatment was started. This consisted of 3 000 I.U of vitamin D orally per day during the first week. After the first week, the dosage was raised to 5.000 I U per day In addition, 1 g of calcium gluconate was given by mouth every day Three weeks after the beginning of treatment serum-Ca and serum P had risen to 9.3 and 5.2 mg per 100 ml respectively Radiographs showed typical lines of Mueller in the rachitic metaphyses (Fig. 1B). Administration of parathyroid extract in the same dosage as before resulted in a fall of serum-P to 3.7 mg per 100 ml. Serum-Ca did not change however (Fig *).

After another * weeks of continued treatment, serum-Ca was 10.3 and serum P 5 4 mg per 100 ml. A third series of

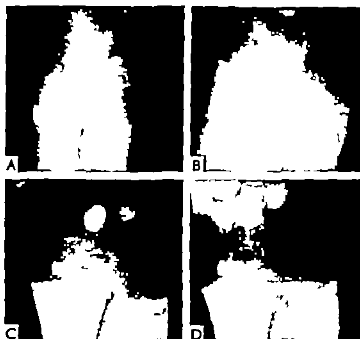


Fig 1 Radiographs of the right wrist A: On day 1 before treatment. B On d ?4, after 3 weeks of treatment C: On da 54, after 7 weeks of treatment D: On day 73, after 9 weeks of treatment

injections of parathyroid extract again caused a significant fall of serum P but did not result in a change in serum-Ca (Fig ?). One week later radiographs showed advanced, though not yet complete healing (Fig 1C). The final attempt was made 8½ weeks after the beginning of treatment Judged by the radiographs, healing was virtually complete at this moment (Fig 1D) This time serum-Ca rose from 10 ? to 12 0 and serum P fell from 5 9 to 4 1 mg per 100 ml after a total of 700 units of parathyroid extract

The same batch of parathyroid extract was used throughout the study (Parathormone Lilly lot number 1010–785405)

Discussion

The data show that in this child and with the moderate dose of parathyroid extract used the phosphate lowering and calcium raising effects of parathyroid extract did not return simultaneously Although the parathyroid glands probably secrete 2 hormones [2] it has been firmly established that only one hormone is responsible for causing hypophosphataemia—by decreased renal tubular reabsorption of phosphate—as well as hypercalcaemia—by a direct action on bone [8]. Most likely therefore the data point to a difference in end-organ response In this respect it is of significance that no structural change occurs in the kidney in rickets whereas bone structure is highly abnormal and is only beginning to be repaired when serum-Ca and serum P have resumed normal level as a result of treatment with vitamin D As shown by the radiograph rickets was far from cured on day ?8 not completely cured on day 56 whereas it was no longer

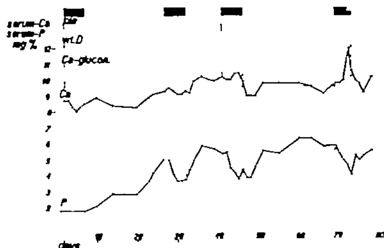


Fig. 2. Effect of administration of parathyroid extract on serum-Ca and serum-P before and during treatment with vitamin D (see text).

visible on day 73. This may imply that the abnormal structure of the skeleton is a factor which prevents parathyroid hormone from causing hypercalcaemia.

Carnes [1] found in hypocalcaemic rachitic rats that the bone matrix could be resorbed regardless of its mineral content as a result of administration of large doses of parathyroid extract (250 U.S.P. units per day for 3 days). He noticed that the extent of the rise of serum-Ca following administration of parathyroid extract depended upon the degree of mineralisation of the skeleton, the higher the degree of mineralisation, the higher the rise in serum-Ca. In florid rickets in human beings mineralisation of bone is incomplete [9]. Therefore it is conceivable that the failure of serum-Ca to rise upon administration of parathyroid extract at a time when the skeletal lesions were still present, was due to quantitatively impaired resorption of incompletely mineralised bone. On the other hand in the experiments of Harrison and his co-workers [3, 4] a failure of parathyroid extract to elevate serum-Ca was found in vitamin D-deficient non-rachitic rats with apparently normal bone structure.

It must be concluded therefore that the mechanisms responsible for these observations are complicated and not properly understood at present. However the data presented serve to emphasize the intricacy of the interrelations between vitamin D and parathyroid hormone in bone metabolism [7]. Until more of the underlying mechanisms is revealed it is necessary to be cautious in ascribing certain metabolic phenomena in rachitic bone disease exclusively either to vitamin D or to parathyroid hormone.

Summary

The effect of administration of parathyroid extract to a patient with severe vitamin D-deficient rickets was investiga

ted. Before treatment parathyroid extract had no effect on serum-Ca and serum P Upon treatment with vitamin D the hypophosphataemic action of parathyroid extract could be elicited at an earlier stage of healing than the hypercalcaemic action

The findings for which a proper explanation cannot be given at present are briefly discussed.

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Possible Factors in the Incidence of Coeliac Disease

by J A. BLACK

This paper is based upon a survey of the incidence of coeliac disease in children born in Glasgow between the beginning of 1941 and the end of 1955 In addition to the recording of the actual number of cases, a detailed analysis of the case records was made to see whether any genetic or environmental factors could be discovered which might affect the incidence of coeliac disease The survey was finished in September 1961 though it would have been desirable to have continued longer in order to include as many cases as possible with a late onset, but as there were only 4 cases in the whole survey with an onset over the age of 6 years it is unlikely that many were omitted on this account. For more complete information on the trend in incidence a further figure was obtained for children born in 1956 which has not otherwise been considered in the analysis.

Few surveys of the incidence of coeliac disease have been made but Davidson & Fountain s [3] study and that of Carter Sheldon & Walker [1] provide some evidence with which to compare the present results. Davidson & Fountain collected data from the special ration returns for cases of steatorrhoea and coeliac disease during the war and the immediate post war period and concluded that there was a higher incidence of coeliac disease in Scotland than in England and that the incidence was highest in the West of Scotland. Carter *et al.* gave an estimate of the incidence of coeliac disease in the London area during the period 1952–March 1958. In neither of these studies is a direct figure for the variations in annual incidence of the disease available Davidson & Fountain gave figures for England and Wales for 1943–1948 and for Scotland for 1940–1948 but as the authors point out, it is probable that many factors, other than purely clinical ones, prompted the completion of a request for special rations for a case of coeliac disease so that the observed fluctuations in applications from year to year cannot be considered as reflecting genuine alterations in the incidence of the disease. Davidson & Fountain regarded the figures for 1946–1948 as being the most reliable and it is these figures which have been used in Table in calculating the incidence for England and Wales, and for Scotland as a whole. The data of Carter *et al.* were analysed primarily from the point of view of family concentration and refer only to the overall incidence for the whole period studied, their estimat is an indirect on based upon comparison with the known incidence of pyloric stenosis

TABLE I

Coeliac disease	Congenital heart disease (control group)	Legitimate live births (Registrar General Scotland [23])
Total number 120	140	
Classes I & II 4 2	1.3	14 6
Class III 5...	59.8	53.
Classes IV & V 42.5	28	31 6

In many respects Glasgow is ideal for a survey of this kind with a population of over a million in which changes due to rehousing outside the city boundaries have been small and contributions to the population from Ireland and from the Highlands and Islands have remained fairly constant during the period under review (Miller & Tivy [11]).

Methods

From a study of the case records it was easy to exclude those living outside the city boundaries at the time of their first attendance at hospital the discrepancy between the address at the time of birth and at the time of hospital attendance was considered to be small. Since there are only two main paediatric units in Glasgow it seemed reasonable to assume that practically all children with coeliac disease would have attended at some stage in their illness either at the Royal Hospital for Sick Children or at the Paediatric Unit of St. ...hill General Hospital, and the records of these two hospitals only were examined.

An attempt was made to record all cases of coeliac disease occurring in children born between the beginning of 1941 and the end of 1955, and resident in Glasgow at the time of their first hospital attendance. The figures so obtained were then considered under the following headings: overall incidence for the whole period of 15 years, and quinquennial incidence for the three 5-year period 1941–45, 1946–50 and 1951–55, expressed as ratio to the total number of births for each year. The annual incidence of coeliac disease in children born in each year including 1954 for this purpose was expressed as the number of cases per 10,000 live births as this gave an easier comparison with other sets of figures (Fig.). Particular attention in the hospital histories was also paid to the recording of the father occupation the early feeding history the age when mixed feeding was introduced the age of onset of symptoms of coeliac disease and its relationship to the start of mixed feeding, and to the presence of any relative affected by coeliac disease or similar disorder. Similar data were obtained from a control group of children with congenital heart disease born in Glasgow over the same period from the records of the Royal Hospital for Sick Children, Glasgow (Table 1). These control cases were randomly placed within groups according to their years of birth until three groups had been built up having the same numbers in each quinquennium as the cases of coeliac disease. In this way it was hoped to eliminate any bias in class structure due to an attendance at hospital.

As a diagnostic criterion a response to gluten free diet would have been desirable but it was clearly impracticable to subject so many cases (131 accepted cases) to a diagnostic period on a gluten free diet when this was no longer indicated, or where older patients had long since ceased to attend hospital and were free of symptoms. It was therefore decided to accept as diagnostic criteria the following points: ( ) clinical: diarrhoea persisting for longer than 4 weeks, with accompanying anorexia, failure to gain or actual loss of weight during the same period and the usual physical findings of coeliac disease (b) confirmation of the clinical criteria by any one of the following (1) stool faecal fat content of 30 or more ( ) a fat retention by balance study of less than 90 (3) a vitamin A curve or thyle... micron count considered typical of coeliac

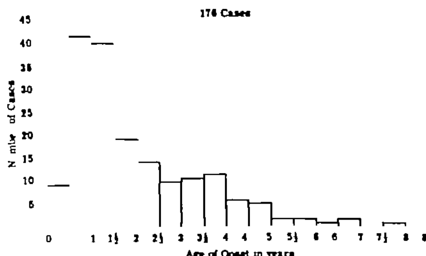


Fig 1

disease (4) a response to a gluten free diet. The response was assessed by an acceleration of gain in weight and height continued during the giving of a gluten free diet for t least 6 months. Stool fat analysis had been done on 129 cases, fat balances on 2 cases, vitamin A curves and chylomicron curves on 3 cases each, and in the remaining 17 cases there was no biochemical confirmation but there was an acceptable response t a period on a gluten free diet. A gluten-free diet was in fact given an adequat trial in 71 cases and failure t respond was recorded in 9 instances in which there was considerable doubt as t adherence to the diet.

Results

Feeding history

The early feeding history was given in 177 cases, of which 37 % were breast fed for * months or longer In the control group (records in 140 cases) only 24 % were breast-fed for the same length of time It is probable that in this instance cases of congenital heart disease are inadequate controls as there is often difficulty in feeding due to dyspnoea or early ad mission to hospital, either of which may interfere with the proper establishment of breast feeding. During the national enquiry into "Maternity in Great Britain" [9] a figure of 33 % for breast feeding for _ months or more was obtained for Glasgow so that it seems unlikely that the incidence of breast feeding in cases of coeliac disease was in any way unusual.

Age of onset (Fig. 1)

Of the 181 cases the age of onset was specifically recorded in 176 instances (3 cases had an indefinite onset and in 2 instances the age of onset was not re corded) Eighty-two cases (46 %) developed continued symptoms between th ages of 6 months and 18 months and in only 9 cases was there a definite onset before the age of 6 months. There were thus 85 cases (48 %) who developed coeliac disease relatively late after the age of 18 months. There was no significant

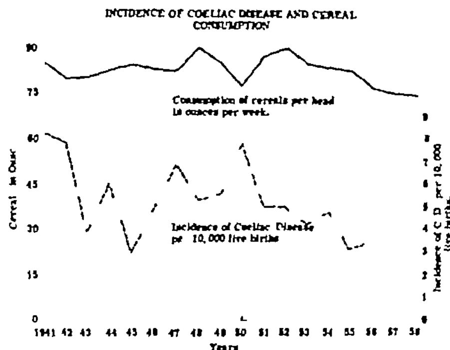


Fig. 2.

difference in the age of onset between the breast-fed and artificially fed groups between the boys and the girls.

Relation t mixed feeding

The average age at introduction of mixed feeding was " * months (controls 6.4 month) the earliest being * months. Seventy three per cent of the children started mixed feeding between the ages of 0 months and one year In 6 cases symptoms started before mixed feeding was introduced, but it i difficult in a retrospective survey to exclude the possibility that cereals were given in the bottle feeds

Sex incidence

There were 9? males and 80 f males giving a ratio of males to females of 1 03 1 which differs little from the expected ratio of 1 06 1 for this period. The average age of onset was the same in both males and females.

Social class incidence

The data for the father's occupation were given in 130 cases. Where more than one case occurred in a family this wa counted a one case All cases where the only record was of war time service were excluded. The incidence in th different social classes (Regi trar General's Classification, taking Classes I and II together and IV and V together) was compared with the control group of chil lren with con genital heart disease which appears t be reasonable representative for the lass structure of the total legitimate births for Scotland (Registrar General for Scotland [23]) The figures given above

TABLE 2.

Region	Date	Incidence	Source
England and Wales	1946–48	1 8120	Davidson & Fountain [3] recalculated
London rea	1952–March	1 2000–	
	1958	1 6000	Carter *et al.* [1]
Scotland	1946–48	1 3584	Davidson & Fountain recalculated
Scotland regions			
East	1943–48	1 5176	
North	1943–48	1 14,624[a]	Davidson & Fountain recalculated
S. East	1946–48	1 3430	
West	1943–48	1 1851	
Glasgow	1941–55	1 1778	
Glasgow	1941–46[b]	1 1773	
Glasgow 5-year periods	1941–45	1 1734	Present survey
	1946–50	1 1608	
	1951–55	1 2061	

The numbers involved are too small t be significant.
[b] Dates of birth as opposed t year of notification, for comparison with preceding figures for West of Scotland 1943–1948.

(Table 1) show a relatively low incidence of coeliac disease in Classes I & II and a high incidence in Classes IV & V

Variations n annual ncidence (Fig *)

The annual incidence expressed as cases per 10,000 live births showed the highest figures for 1941 with a slight falling off in 1943–45 then an increase for 1947–50 followed by a fairly steady fall till 1956 the last year recorded.

Since one of the major alterations af fecting the civil population during the wars and immediate post war period was in the diet, the figures for the annual incidence of coelia disease have been plotted on the same graph as those for the average weekly consumption of cereal products for each year (Ministry of Food [1 –15]- Ministry of Agriculture Fisheries and Food [16– 1]) There is, however no good relationship between the two sets of figures, apart from the fact that both the incidence of coeliac disease and the consumption of cereals are declining

The ncidence n Glasgow compared to that n oth areas (Table 2)

The overall incidence for the period 1941 to the end of 1955 was 1 case per 1778 live births, or for each quinquennial period, 1941–45 1 case per 1734 live births 1946–50 1 case per 1608 live births and for 1951–55 1 case per 2061 live births. Since the only figures with which these can be compared are those given by Davidson & Fountain [3] for the ration returns for the West of Scotland for the years 1943–48 the survey figures have also been calculated, as shown in T ble –, for the period 1941–46, referring in this case to the years of birth. The two sets of figures show a reasonable correspondence suggesting that the methods used are probably reliable and that the very high incidence in the West of Scotland

TABLE 3. *Cereal consumption in ounces per head per week*

Year	Class A	Class B	Class C	Class D
1951	71.9	83.5	86.8	83.5
195[illegible]	[illegible]4.2	83.1	80.9	88.4
1953	69.3	78.9	85.6	87.[illegible]
1954	70.4	[illegible]8.3	84.0	84.5
1955	72.1	78.6	83.9	82.8
1956	69.1	75.3	79.4	77.8
19[illegible]7	65.1	73.6	75.1	[illegible]8.1
1958	60.4	71.9	75.7	74.4

1. These classes correspond roughly to the Registrar Classes as follows, I(A), II(B), III(C), IV & V(D)
2. 1951 1952 (Ministry of Food, 1953 and 1954) 1953–1958 (Ministry of Agriculture Fisheries and Food, 1955 1956, 1957 1958–1960)

(Glasgow can be considered as representative of the West of Scotland) compared to other parts of Scotland and to England and Wales is correct

The relatively low incidence in the Eastern part of Scotland makes this area more comparable to England and Wales and it is probably relevant that this is a wheat-growing area while in the West of Scotland and also in the North Western region of England wheat has never been grown in any significant amount and oats have always been the predominant cereal. This is considered further in the discussion.

Discussion

In this survey in contrast to most other series (Hardwick [9] Carter *et al* [1]) coeliac disease did not appear to be commoner in girls than in boys and it does not seem likely that sex differences are an important factor in the development of the disease Nor was there evidence of any difference in the incidence or age of onset between those children who had been breast fed and those who had been artificially fed so that the type of feeding during infancy also seems unimportant

In considering the great variation in the age of onset of coeliac disease it is clear that in many cases symptoms did not appear until some years after the introduction of wheat cereal products into the diet There must therefore be other factors, apart from the consumption of wheat products which determine the development of coeliac disease at least in a proportion of cases. Infections, such as infantile gastroenteritis may play some part in precipitating the onset of coeliac disease but there is no direct evidence on this point though it is true that the incidence of infantile gastroenteritis has declined greatly since 1947 as judged from the number of reported deaths (*Medical Officer of Health Glasgow* [10]). In a retrospective survey it is in any case impossible to distinguish with any certainty between gastroenteritis and an onset of coeliac disease associated with severe diarrhoea and vomiting as in a coeliac crisis

Another possible explanation for the cases of coeliac disease with a late onset is that some children can tolerate small amounts of gluten in their diet and develop symptoms only when they take large amounts. This suggests that there *hould be an increased incidence in a com*munity which consumes large amounts of wheat products The increased incidence in social classes IV & V which have a greater average consumption of cereal products than the higher income groups (Table 3) would tend to confirm this view though the classifications used by the Registrar General and the Ministry of Food are not strictly comparable and no figures are available before 1951

Though the figures obtained in this

survey suggest a relatively high incidence of coeliac disease in the West of Scotland, there is as yet insufficient comparable information about other parts of Great Britain for one to reach any conclusions which would throw further light on the factors which may govern geographical variations in the incidence of this disease

Summary

A survey of the incidence of coeliac disease in Glasgow between 1941 and 1955 is presented, and certain factors relating to the incidence and onset of coeliac disease are discussed.

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TABLE 1 *Certain and probable cases of Pendred's syndrome reported*

Authors	No. of families	No. of cases
Baschieri *et al.* (1961)	1	2
Batsakis & Nishiyama (196-)	4	6
Brain (1927)	5	1
Decourt *et al.* (1962)	1	2
Deraemaker (1956)	1	2
Elman (1958)	1	4
Fishman *et al.* (1960)	1	4
Fraser, Morgans & Trotter (1961)[b]	7	112
Friedlieb (1951)	5	5
Harnack von, Horst & Lenz (1961)	3	9
Hopkins & Guilder (1949)	?	8
Johnsen (1958)		12
Kontras *et al.* (1960)	1	2
Lindenov (1945)	?	18
McGirr, Hutchison & Clement (1959)	3	5
Pendred (1896)	1	2
Thieme (1957)	1	4
Thould & Scowen (1961)	?	23
		228[d]

[a] Including four cases from two families earlier reported by Elman and Thieme respectively.

[b] Including five cases from two families earlier reported by Brain.

[c] Occurrence of goitre in large materials of congenital deafness.

[d] Excluding cases reported twice.

Butanol-extractable iodine (BEI) was done according to the method described by Man, Kydd & Peters [22].

Thyroid uptake tests were done by the conventional technique. Amberlite IRA Resin 400 was used for the separation in the determination of PBI¹³¹.

Perchlorate test. I¹³¹ (or I¹³² on some occasion) was administered orally with the patient in the fasting state. The activity over the neck was measured during 1-min. periods at intervals of 10–15 minutes. After one hour the subject was given 200–400 mg of potassium perchlorate dissolved in water and the thyroid activity was followed for a further hour. The values obtained were not corrected for extrathyroidal activity.

Hearing studies

Pure-tone audiometry was performed with an Amplivox audiometer. The hearing defect was located with the aid of recruitment test including the stapedius reflex test and Békésy audiometry.

Results

Incidence

In the medical examination of 90 deaf children at the three special schools mentioned above four (Cases 9–1[illegible]) were found to have goitre, all being cases of Pendred's syndrome. In one (Case 9) the disorder had been known previously. These three schools serve the provinces of Skåne, Blekinge, Småland and Öland in southern Sweden with a total population of about 1.7 million, of which about 234 000 are of school age (7–15 years). The proportion of school children at special schools for deaf children is thus about 0.04 %. The number of school children with hearing defects but attending ordinary schools is about three times as large as those at special schools; these children were not examined.

The number of known cases of Pendred's syndrome in school children is five including one (Case 8), the sister of Case 9, who is attending an ordinary school but in a special class for children with hearing defect. The minimum incidence of Pendred's syndrome in school children in this region is thus roughly 1:40 000.

Familial history

There were seven single cases and five familial cases, the latter distributed between two sibships (Table 3). The families with a single case were small with only one or two children in the family

Table 2 *Clinical data in 12 patients with goitre and hearing loss*

Case	Sex	Age in years	Height/ weight cm/kg	Age in years at recognition of: Hearing defect	Goitre	Character of goitre	Hypothyroidism	Course and remarks
Y L.	F	9	138/37	Infancy	9	Moderate smooth	0	Diagnosed as Pendred' syndrome t 9 yrs. but cytological aspiration biopsy at 11 yrs. of age revealed lymphocytic thyroiditis as the cause of the goitre. No therapy yet.
.S.F	F	13 14	146/33 154/38	Infancy	9	Large nodular adenoma lobe	0 +	Referred as (?) thyrotoxicosis. Six months later she felt tired and cold. Symptom-free and disappearance of goitre on 0.15 mg thyroxin.
A.P	F	14½	164/44	Infancy	11	Large nodular	0	Goitre disappeared on 0.2 mg thyroxin but recurred yrs. later Decrease of goitre on thyroxin 0.25 mg.
G O	M	10½ 12	 148/38	Infancy	cong.	Moderate, a little nodular	0	Goitre revealed at follow up of neonatal goitre. Unchanged status for 2 yrs. without therapy
5 A. A.	F	16 28	 168/ 0	Infancy	?	Large highly nodular	0 +	Strumectomy t 16, 22 and 26 yrs. of age. Myxedema after the last operation. On thyroxin no hypothyroid symptoms and no recurrence of goitre.
6. S S.	M	15 22	 167/63	?	cong	Large nodular adenoma L lobe	0 +	Strumectomy t 15 and 24 yrs. of age. Hypothyroid after last operation, thyroxin was instituted, well since then.
7 A. S.	M	14 31	 172/64	?	?	Large smooth	0	Strumectomy at 14 and 20 yrs. of age Total thyroidectomy at 28 yrs. due to suspected malignancy Histopathologic diagnosis of papillary thyroid cancer Is well on thyroxin 3 yrs. after operation.
R W	F	11	142/29	?	1	Large smooth	0	On thyroxin, regression of goitre.
M W	M	6	124/28	Infancy	?	Large smooth	0	Slight mental retardation, IQ 80. Abnormal EEG Goitre regression on thyroxin.
I K	F	13	161/65	?	11	Large smooth	+	Moderate hypothyroidism. Normal skeletal age. Behavior abnormal. IQ < 70. EEG abnormal. On 0.2 mg thyroxin no hypothyroid signs and decrease of goitre
I P	F	12	152/47	Infancy	10	Large slightly nodular	0	On 0.2 mg thyroxin regression of goitre but the thyroid still enlarged after treatment for one year
B R.	F	14	158/50	Infancy	11	Large nodular adenoma lobe	0	After 7 months on 0.2 mg thyroxin only small adenoma in the right lobe palpable

TABLE 3. *Family histories in 1° patients with goitre and hearing loss. Cases No. * 1 Pendred's syndrome*

Family no.	Case	Sex	No. of sibs order in sibship	Goitre and hearing loss: Parent	Goitre and hearing loss: Other rels.	Goitre only: Sibs	Goitre only: Parents	Goitre only: Other rels.	Hearing loss only: Sibs	Hearing loss only: Parent	Hearing loss only: Other rels.	Remark
I	1	F	/	–	–	–	+	+	–	–	+ +	The patient had chronic thyroiditis. Mother goitre in adolescence. A maternal relati operated for th ro toxicosis. Hearing defect in four maternal relat es
II		F	/2	–	–	–	–	+	–	+	–	Parent first cousins. F er hearing defect fter mumps. Paternal aur goitre
III	3	F	1/	–	–	–	–	+ +	–	–	–	Three m ternal rela c goitre (1 thyrotoxicosis
IV	4	M	/2 (half-sib)	–	–	–	–	–	–	–	–	
V	5	F	/6									Brother di d 1 1 8 yrs of age in an accident; treated f thyroid disease but hearing unknown.
	6	M	5/6	–	–	–	–	–	–	–	–	
	7	M	6/6									
VI	8	F	1/	–	–	–	+	+ +	–	+	+ +	See special pedigree Fig 1
	9	M	/2									
VII	10	F	1/1	–	–	–	+	–	–	–	–	Mother operated for th ro toxicosis.
VIII	11	F	1/	–	–	–	–	–	–	–	–	
IX	12	F	1/1		–	–	–	+ +				One paternal relativ ew maternal relativ operated for thyrotoxicosis. Thre maternal rel operated for large goitres

The parents of one patient were first cousin There wa no endemic goitre in the area where the patient lived The combination of goitre and deafness occurred in only one generation On the other hand goitre without deafness was rather prevalent in previous generations and wa present in five of the eight families with Pendred's syndrom and several members of three of the families had goitre (Table 3). Thyrotoxicosi had been diagnosed in four and the other relatives probably had non toxic goitres a ailable information was not sufficient to warrant a firm diagnosi One of th single cases (Case 1) had not Pendred syndrome but lymphocytic thyroiditi The incidence of goitre in the family of this girl d d not differ from that in th rest of tl material.

Other types of hearing defect oto-sclerosis and presbyacu is occurred in one family the pedigree in Fig 1 Apart from this family and a father with acquired hearing defect and belonging to another family there were no relative f patient with Pendred's syndrome with known

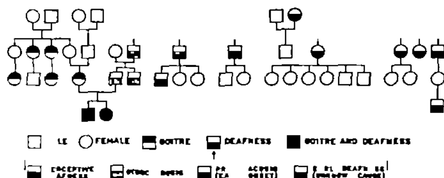


Fig 1. Pedigree of Family VI.

hearing defects On the other hand, maternal relatives of the girl with thyroiditis probably had a hereditary type of hearing loss, but the information was too meagre to be conclusive

Hearing loss and hearing tests

The hearing defect was suspected during the first 2 years of life and was probably congenital. Judging from the histories of a few cases hearing had been somewhat better during the first year of life than later Most of the patients had a limited vocabulary and speech difficulties.

The hearing loss was perceptive greater for high tones than for low ones. The results of the hearing tests are given in Table 4 and the audiograms in Fig _. The audiograms showed symmetrical lesions though with small side differences in some patients Five of the Pendred cases were deaf In some cases the hearing defect was almost complete on one side and partial on the other The hearing defect in the three adult sibs was moderate but

with thyroiditis did not differ from that in the Pendred cases.

Recruitment was demonstrable in four cases.

Goitre and thyroid status

The goitre was often first noticed at about 10 years (Table 2) In two cases the goitre was visible at birth. In one of them the goitre regressed spontaneously during the first year of life and was thought to

TABLE 4 *Hearing tests.*

Case	Average hearing loss in db at 500–2000 cps		Recruitment
	right	left	
1	Deaf	Deaf	–
2	Deaf	67	+
3	73	Deaf	+
4	Deaf	Deaf	–
5	35	43	
6	25	30	Not investigated
7	45	33	+
8	Deaf	88	
9	65	Deaf	
10	Deaf	Deaf	Not investigated
11	[illegible]	[illegible]	

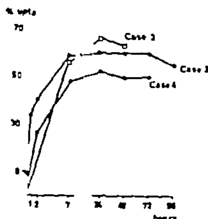


Fig. 3. Iodine upt ke tests in Cases -4 showing the rapid accumulation of radioiodine

of three cases with Pendred s syndrome The accumulation of radioiodine was rapid and the maximal uptake abnormally large The conversion rate in these three cases was high and PBI[131] was raised in two and normal in one A characteristic positive perchlorate response is illustrated in Fig 4 The range of variation of the outcome of the perchlorate test wa remarkably wide the decrease in uptake ranging from about ?0 (excluding the value of the patient not in fasting state) to 0 average 3? Perchlorate tests performed in the parents of the family VI were normal

Pathol gy

The surgically removed goitres had a nodular character Microscopically the follicle lining cell were high and of active appearance The follicles were often small and contained scanty or no colloid The epithelium sometimes howed nuclear polymorphism and papillary infoldings However a papillary tumor was not suspected except at the la t trumectomy in Case ? when nodules of thyroid tissue were found cranial to the thyroid mass These nodules had mainly the same microscopic appearance as the goitre itself but the papillary changes were more pronounced. The nodules were interpreted as lymph node meta tases of a highly differentiated papillary thyroid cancer (Fig 7), but on check examination of the hi topathologic section which was prompted by the clinical diagnosis of Pendred s syndrom the picture wa considered not to be malignant No blood vessel invasion could be seen The epithelial cells had a high degree of maturity and showed no mitoses No metastases were found in th numerou lymph nodes which were removed at the neck dissection

Other signs and symptom

Mild to moderate mental retardation of unknown cause wa noted in two of the cases The perinatal history wa non contributory The electroencephalogram showed unspecific abnormalities

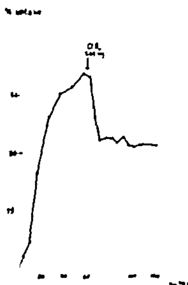


Fig 4 A characteristic perchlorate response in Pendred syndrome (Case



Fig. 8. Histologic appearance of the goitre at the last strumectomy in Case 7. *A* The goitre has papillomatous structure similar t that of highly differentiated papillary thyroid carcinoma. 25 *B* The epithelial cells have highly regular appearance as in papillary adenoma. 104.

Therapy nd course

Substitution therapy was necessary in the sibs with recurrent goitres and in the to prevent further growth of the goitres, such treatment was also indicated in the euthyroid subjects with an iodination

Individual data on the response to therapy and the further course of the disease are given in Table 2. Thyroxin led to a rapid and marked decrease of the goitres, some disappeared almost completely. In the adult sibs hearing was somewhat better than when they were in the severely hypothyroid state, but otherwise thyroxin had no effect on the hearing ability.

Discussion

Diagnosis

In any area without endemic goitre the diagnosis of Pendred's syndrome should be considered in every patient with goitre and hearing loss. The presence of deafness combined with goitre in sibs strongly supports the diagnosis. In single cases the diagnosis has to be proved by a positive perchlorate test and a hearing test showing a perceptive type of hearing loss. It might otherwise be an incidental association of goitre of any cause and hearing loss as in Case 1, in which lymphocytic thyroiditis occurred in a girl with early deafness. However, in view of the wide range of variation of the results of the perchlorate tests a negative perchlorate test would not exclude Pendred's syndrome with certainty. *Fraser, Morgan & Trotter* reported the decrease of thyroid activity to range from 15 to 80 and occasionally to give equivocal results (13). A thyroid biopsy may sometimes be necessary to exclude the possibility of a coexisting hearing loss and Hashimoto's disease in which a positive perchlorate test may be encountered (4). Pendred's syndrome is distinguishable from endemic cretinism with goitre and deafness by the absence of cretinoid signs by positive perchlorate test and by its appearance as a recessive inherited disease in areas without endemic goitre.

There were seven single cases in the present material. Six of them had a positive perchlorate response and were diagnosed as Pendred's syndrome. The remaining case was at first interpreted as Pendred's syndrome but as the perchlorate response was normal thyroid biopsy was done and revealed diffuse lymphocytic thyroiditis. Since no thyroid biopsy was done in the other single cases and since no search was made for thyroid antibodies the diagnosis of Pendred's syndrome was not quite unobjectionable though very probable. There was, however, no reason to doubt the diagnosis in the girl with parental consanguinity and in the boy with neonatal goitre. The high incidence of single cases in our material seems to be due to the small number of sibs (Table 3).

Perchlorate tests were not performed in the three adult sibs because they were undergoing treatment with thyroid hormones. The early onset of goitre in sibs, repeated relapses of goitre after strumectomies and the microscopical appearance of the goitres were evidence of some disturbance of the synthesis of thyroid hormones, an error presumably of the same character as in other cases of familial nonendemic goitre and hearing loss.

Incidence

The incidence of four cases of Pendred's syndrome among [illegible] deaf school children stresses the necessity of careful examination of the thyroid in deaf children. The minimum estimate of the frequency of Pendred's syndrome in school children in southern Sweden, roughly

1/50 000 is in good agreement with Trotter s assessment of 1.5–3 0 per 100 000 inhabitants.

Genetics

The inheritance of Pendred s syndrome is due to an autosomal recessive gene [8 1 37] The occurrence of consanguinity between unaffected parents reported previously in three families [1 8 L] and in one family in the present material is consistent with this mode of inheritance. In Deraemaker s family three out of 1 sibs were affected [8] an expected distribution for a recessive disorder In two families the syndrome has appeared in more than one generation, but in these families both parents had the disease [12, 19] The offspring of one family had congenital hypothyroidism [18], which is difficult to explain from a pure genetic point of view but might be ascribed to an aggravation of the condition by intrauterine factors secondary to the mother s thyroid dysfunction. In the Danish family reported by Johnsen [19] goitre and deafness appeared not only in the parents and their children, but also in two offspring of one of the children. This suggests the possibility of another mode of inheritance in this family which was atypical also in other respects, in that most affected members had low PBI levels and some were clearly hypothyroid.

The strong association of goitre and hearing loss in affected sibs has been taken as evidence for the existence f a single gene defect or of a closely linked double gene defect responsible for the thyroid dysfunction and the hearing defect [1., 37] On the other hand, thyroid disease without hearing loss was fairly common among relatives in earlier generations though nothing suggested that the thyroid dysfunction was of the same type Perchlorate tests in presumed heterozygotes have been negative [13] as in the parents of one family studied in the present material. The occurrence of thyroid disorders in relatives is common also in other kinds of thyroid disease which indicates the existence of a genetic factor of varying and often reduced penetrance However Pendred s syndrome presents itself clearly as a recessive It seems that the presence of such a recessive gene causing a distinct thyroid disorder in homozygotes would also favour the development of other kinds of thyroid disease in heterozygotes

A positive perchlorate or thiocyanate test is characteristic of one particular type of familial goitrous cretinism [15 26 30 33] in which almost all activity is discharged from the thyroid upon blocking of the thyroid trapping mechanism, and in which in contradistinction to Pendred s syndrome the thyroid has no ability to synthetize thyroxin [15 24]. As both diseases are transmitted by recessive genes, their gene defects cannot be identical because of the clear clinical and biochemical differences between them.

Positive perchlorate tests have been found not only in Pendred s syndrome and in the familial goitrous cretinism discussed, but also in some cases of Hashimoto s thyroiditis [24], in deaf-mutes without goitre [1], and in some patients with colloid nodular goitre and a history of goitre in the family [6 11]. The pathogenesis of the iodide discharge in Hashimoto disease is obscure but is probably acquired. The response in deaf mutes

without goitre need further investigation of their thyroid function and confirmation of the finding in other geographical area.

The thyroid dysfunction in those goitrous patients with a strong family history of goitre and a positive thiocyanate test [0 11] cannot be distinguished from that in Pendred's syndrome. This may indicate that one and the same gene defect is responsible for the thyroid dysfunction in both conditions and thereby support the hypothesis of a double gene defect in Pendred's syndrome. However th biochemical processes necessary for the iodination in the thyroid are not properly understood [25] and there may be a variety of abnormalities capable of producing a positive perchlorate or thiocyanate test. In addition the thiocyanate test was positive in consecutive generations in one of the families reported [11] and in another family there was a substantial disproportion between PBI and BEI [0].

Pathology

The often intense hyperplasia of the follicular epithelial cells has aroused suspicion of malignancy and thyroid carcinoma has sometimes been considered [0 ?? 35]. In his study of this problem Smith however could not find reliable sign of carcinoma in any of these cases and indeed there is still no case of Pendred syndrome in which thyroid changes considered malignant has given rise to metastases. The difficulties in the histologic diagnosis is documented by the findings in Case 7 of the present material.

The intense follicular hyperplasia is probably due to an increased secretion of

in thyroid hormone synthesis. Indirect evidence of the goitre provoking effect of TSH i th rapid regression of goitre on substitution therapy with thyroid hormones.

The auditory organ

The hearing defect was of the same type in all our cases but it varied in severity in that it was milder in family A than in the rest of the present material. This is in accordance with Fraser, Morgans & Trotter [1] who found the extent of the hearing loss to range from total deafness to losses which could be corrected by use of hearing aid.

The demonstration of the recruitment phenomenon in four cases indicates that the lesion in these cases is localized to the cochlea. In normal subjects or in patient with a hearing defect due to retrocochlear lesions the loudness with which the tone is perceived varies with the intensity of the tone. Cochlear lesions on the other hand are characterized by a disproportionately high increase of the loudness with intensity. This recruitment was demonstrable by measuring the difference in the threshold limen necessary for registration of changes in the intensity of the sound by Békésy audiometry and by examination of the elasticity of the stapedius reflex. The recruitment test are unreliable in very severe hearing losses as in Cases 1 and 4. The pathogenesis of the cochlear lesion is unknown but it is apparently not secondary to thyroid hypofunction.

The vestibular organ also seems to be affected since the response to caloric stimulation has been found to be diminished [18, 19, 10].

Thyroid function

In the normal thyroid anions such as ClO and SCN blocking the iodide-concentrating mechanism of the thyroid [38 39] causes no measurable decrease of thyroid radioactivity at radioiodine tests, the amounts of free iodide in the thyroid being very small owing to a rapid in corporation of iodide into organic linkage [5] The perchlorate response in Pendred syndrome demonstrates an outflow of considerable amounts of labelled iodide from the thyroid This means that the thyroid content of free iodide was abnormally large indicating that the rate of organic binding of iodine was abnormally low [25] The underlying error is unknown. One theory supposes the existence of an extrathyroidal metabolic block leading to accumulation of substances interfering with both thyroid function and the auditory organ [1,] There is still no evidence for this theory [37] though the remarkably wide variation in the results of the perchlorate tests might be ascribed to differences in amounts of substances interfering with the iodination process. Another theory supposes a partial peroxidase deficiency [26], but a relative enzyme deficiency can hardly be accepted in homozygotes, especially as loss of peroxidase function satisfactorily explains the total inability to incorporate iodine into organic compounds in the familial goitrous cretinism discussed previously A third theory assumes a deficiency of some other substance (enzyme?) influencing the rate of the iodination process.

Little is known about the turnover of iodine in the thyroid In view of the relative defect in organic binding of iodine one might xpect the conversion rates to be subnormal. However the ability to accumulate iodine is not decreased but increased, as shown by the rapid and early increased uptake of iodine in radio-iodine tests. This is probably a thyrotropin effect. It leads to a rapid disappearance of iodine from the blood giving a high con version rate in persons with only partial impairment of thyroxin synthesis The few PBI[131] determinations have given normal or moderately increased values [16 20 23]. The latter indicate that the turnover of incorporated iodine is in creased, probably owing to intrathyroidal iodine deficiency secondary to the defect in the organic binding of iodine Another possibility implicates leakage of nonhormonal iodinated compounds from the thyroid, a mechanism probable in a few cases [16] but hardly usual in Pendred syndrome

Summary

Twelve patients with goitre and hearing loss were studied. In one girl the goitre was due to chronic lymphocytic thyroiditis and her deafness was familial. The other cases were diagnosed as Pendred s syndrome Eight cases were seen in children belonging to seven families, and three in adults from one family The parents of one girl were first cousins. The goitre was neonatal in two but otherwise appeared in early or middle childhood. The hearing loss was detected during the first * years of life It was perceptive in type and severe in six cases deafness was complete Recruitment phenomenon was demonstrable in four cases suggesting a lesion of the cochlea. Thyroid function was characterized by a defect in the organi binding of iodine as shown by decreased

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Metabolic Alkalosis in Hypertrophic Pyloric Stenosis: Clinical Significance and Treatment[1]

by POUL KILDEBERG

The physiological neutrality regulating mechanisms, operative under normal as well as pathological conditions, tend to establish and secure levels of extracellular and intracellular acidity within the range tolerated by the organism. Large pH deviations in either direction may cause the death of the individual, possibly by interfering with enzymatic metabolism, but in clinical cases it is usually not possible to ascribe to any specific disturbance of acid-base metabolism a specific symptomatology, such disturbances being nearly always secondary phenomena accompanied by more or less complicated disorders of the whole water and electrolyte exchange.

However, one single outstanding exception to this rule is the constant hyperventilation of acidotic patients which for many years has proved a valuable diagnostic sign in the recognition of such conditions as coma diabeticum and uremia. Hypoventilation in metabolic alkalosis, though since long recognized in infants suffering from hypertrophic pyloric stenosis [1, 13, 16], is a clinically less conspicuous feature of neutrality regulation and has not until recently contracted special attention. However, the physiological significance of this phenomenon has been amply verified [18, 22] and its possible bearing upon risk and prognosis in pyloric stenosis will be considered below. Tetanic convulsions (gastric tetany) often experienced under experimental conditions [11, 22, 24] appears to be a relatively rare occurrence in clinical alkalosis. Furthermore, attempts have been made to define renal disorders due to metabolic alkalosis [4, 7, 23, 40], but no clear distinction appears to have been made between functional renal changes, secondary to an altered fluid-electrolyte status, and permanent renal damage, and the question of specificity has mostly been left unanswered. In his large survey of clinical acid-base abnormalities Miller [**] found no evidence suggesting that these disturbances should give rise to any particular single symptoms, except that a reduced pH is accompanied by hyperventilation.

Because of these relationships, and because increasing severity of acid-base disturbances is very often paralleled by increasing dehydration and corresponding aggravation of other electrolyte disorders present, it is difficult to evaluate the cl[illegible]

[1] This investigation was supported by grants from "Fonden for lægevidenskabens ... Arbejde" ... Fyens Stift.

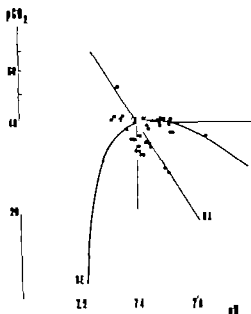


Fig. 1. Disturbances of neutrality regulation occurring in infants and children admitted because of vomiting.
Black dots. Values obtained on admission from 23 infants with pyloric stenosis (diagnosis confirmed at operation in 26 cases).
Open circles. Values obtained on admission from 48 patients resulting from other causes (including dyspepsia, pyuria, nephropathia, diabetic acidosis, cyclic vomiting, rumination, adrenal insufficiency, duodenal obstruction, ileus, epilepsia, encephalitis, and unidentified causes). The two patients showing the highest base excess values suffered from duodenal obstruction (malrotation).
BE = base excess, mEq/l. NA = normal CO_2 absorption line

cal significance of acid-base complications and to define strictly the indications for specific treatment. Whereas the bicarbonate treatment of metabolic acidosis has been a clinical routine measure for many years the treatment of metabolic alkalosis with ammonium chloride to be described below is probably not very commonly used.

In previous papers [18, 19] observations applying to the physiological aspects of metabolic alkalosis were presented, based upon a study of *0 consecutive cases of hypertrophic pyloric stenosis admitted during the years 1961 and 196_ to the Pediatric Department, Odense County and City Hospital. In the present article experience gained from this same case material (including 8 later patients) concerning the treatment with ammonium chloride as well as observations relating to other clinical problems will be described.

Methods and Case Material

The acid-base determinations were carried out using the Astrup micro equipment [35, 37, 38]. For a description of the analytical procedure and the case material, the reader is referred to the aforementioned paper [18] and to Table 1.

To illustrate the course of acid-base parameters a standard diagrammatic scheme was worked out according to the principle proposed by Siggaard Andersen [36] but modified in that the base excess is placed upon the linear scale instead of the pH. In this diagram, deviations of the pH from the line of neutrality approximately equal the sum of the simultaneous deviations of the base excess and the pCO (cf. Fig. *). Base excess (BE) denotes the titratable acid (negative values) or base of the blood. All base excess values have been recalculated according to the revised base excess curve published by Siggaard Andersen [37].

Results

Diagnosis

Fig. 1 gives acid-base data obtained on admission for 23 infants with pyloric stenosis and 48 patients vomiting conspicuously from other causes. All patients with pyloric stenosis presented with a positive base excess value. The figure demonstrates that metabolic alkalosis is by far more pronounced in pyloric stenosis than in the case of low obstruction or nonobstructive vomit

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Metabolic Alkalosis in Hypertrophic Pyloric Stenosis Clinical Significance and Treatment[1]

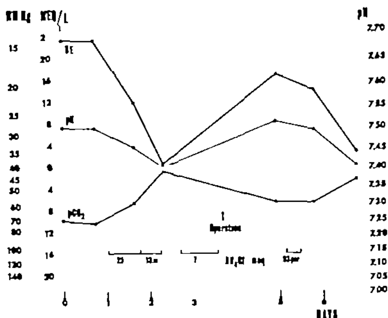
by POUL KILDEBERG

The physiological neutrality regulating mechanisms operative under normal as well as pathological conditions tend to establish and secure levels of extracellular and intracellular acidity within the range tolerated by the organism Large pH deviations in either direction may cause the death of the individual, possibly by interfering with enzymatic metabolism, but in clinical cases it is usually not possible to ascribe to any specific disturbance of acid base metabolism a specific symptomatology such disturbances being nearly always secondary phenomena accompanied by more or less complicated disorders of the whole water and electrolyte exchange.

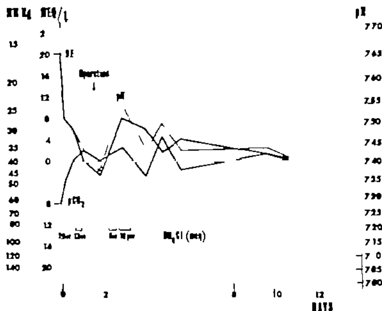
However one single outstanding exception to this rule is the constant hyperventilation of acidotic patients which for many years has proved a valuable diagnostic sign in the recognition of such conditions as coma diabeticum and uremia Hypoventilation in metabolic alkalosis though since long recognized in infants suffering from hypertrophic pyloric stenosis [1 13 16] is a clinically less conspicuous feature of neutrality regulation and has not until recently contracted special attention. However the physiological significance of this phenomenon has been amply verified [18 32], and its possible bearing upon risks and prognosis in pyloric stenosis will be considered below Tetanic convulsions ('gastric tetany') often experienced under experimental conditions [11 22, 24] appears to be a relatively rare occurrence in clinical alkalosis. Furthermore attempts have been made to define renal disorders due to metabolic alkalosis [4 7 23 40] but no clear distinction appears to have been made between functional renal changes, secondary to an altered fluid-electrolyte status, and permanent renal damage and the question of specificity has mostly been left unanswered. In his large survey of clinical acid-base abnormalities Møller [27] found no evidence suggesting that these disturbances should give rise to any particular single symptom except that a reduced pH is accompanied by hyperventilation.

Because of these relationships, and because increasing severity of acid base disturbances is very often paralleled by increasing dehydration and corresponding aggravation of other electrolyte disorders present it is difficult to evaluate the clini-

[1] This investigation was supported by grant from Fonden for lægevidenskabeligt Arbejde i Fyens Stift



c.



d.

b. Case 26. Not Efficient renal compensation during the first pre-operative day probably due to sufficient administration of sodium and potassium, cf. urine figures and discussion p. 140. NAE = net acid excretion in mEq.

Case 15 Note: Well-developed respiratory compensation and marked postoperative alkalosis.
d. Case 14.

TABLE 1

Case	Sex	Age weeks	Birth weight kg	Weight on adm. kg	Duration of vomit. weeks	Base excess mEq/l	S-Na mEq/l	S-K mEq/l	S-Cl mEq/l	S-Urea mg/100 ml	Pylorotomy
						Blood values on admission					
1	♂	3	3.7	3.0	3	+17.1	144	4.4	87		+
2	♂	3	3.4	3.2	<1	+1.3	120	4.6	96		+
3	♂	6	–.0	2.6	<1	+5.4	141	5.9	105		+
4	♂	5	2.6	3.1	<1	+7.0	140	4.4	10*		
5	♂	4	2.9	2.7	<1	+2.9	142	4.6	106	37	
6	♂	8	2.9	3.0	4	+6.1	137	3.7	106	41	+
7	♂	6	3.0	3.0	3	+4.9	137		101		+
8	♂		2.8								
9	♂	5	–.7	2.3	2	+9.6	133		82		+
10	♀	7	3.4	3.4	2	+4.9	153	4.0	92	65	+
11	♀	8	3.2	3.0	1	+10.1	142	3.3	4	114	+
12	♀	3	3.7	3.3	1	+8.3	141	4.8	87	33	+
13	♂	5	2.8	3.3	1	+6.3	136	4.3	88	34	+
14	♂	3	3.5	2.9		+20.1	136	3.3	78	31	+
15	♂	4	3.1	–.6	1	+23.0	133	4.2	72		+
16	♀	5	3.7	2.9	2	+14.9	135	4.5	74	140	+
17	♂	12	–.6	4.7	8	+17.9	131	2.7	76	47	+
18	♀	5	3.0	3.0	1	+4.2	139	4.7	102	18	+
19	♂	10	4.0	5.1	4	+15.0	139	3.5	83	58	+
20	♂	5	2.5	2.7	1	+9.8	143	5.0	92	83	
21	♂	4	3.4	3.3	1	+5.4	143	4.7	96		+
22	♀	7	3.0	3.0	1	+7.6	144	4.4	97		+
23	♂	3	3.7	–.9	3	+10.2	137	4.3	97		+
24	♂	1	3.1	2.8	<1	+7.2	130	5.7		53	+
25	♂	6	4.0	4.0		+17.6	146	3.4	95	70	+
26	♂	2	3.5	3.1	1	+8.1	143	4.8	97		+
27	♂	6	3.5	2.9	3	+9.4	162	2.7	104	73	
28	♂	3	2.9	3.0	3	+3.9	133	5.7	111		+

Remarks. Case 1. Convulsions, see text. *Case 8.* Postmature male infant, transferred from the nursery of the Obstetric Department. Symptoms developed during stay in hospital. The patient recovered without pylorotomy. *Case 9.* Morbus cordis congenitus (ventricular septal defect). *Case 11.* Cf. Fig. 2d. *Case 13.* Cf. Fig. 2e. *Case 16.* Continued hemorrhagic vomitings after pylorotomy. Death from sepsis 4th. day postoperatively. Autopsy showed, besides a hypertrophic pylorus and an adequate pylorotomy, a dilated, inflamed esophagus, a thin-walled esophageal diverticle (diameter 15 mm), and purulent phlebitis. *Case 17.* Complicating pyuria. Pyelograms normal. Cf. Fig. 2a. *Case 25.* Cf. Fig. 2b.

ing. Mild alkalosis may result from vomiting in dyspepsia, diabetic ketosis, pyuria, duodenal obstruction or from cyclic vomiting. Vomiting in renal and adrenal insufficiency was associated with metabolic acidosis. Thus in the case of diagnostic difficulties, significant metabolic alkalosis may be taken as additional evidence in favour of pyloric stenosis, which probably reflects the fact that only in the presence of gastric retention is protracted vomiting likely to remove from the organism a major fraction of the total gastric output of H.

Rather severe secondary metabolic alkalosis has been found to prevail temporarily in patients recovering from compensated respiratory acidosis when the

fall in pCO_2 has preceded the renal adjustment, but this type of alkalosis is not likely to present special clinical problems and is as a rule easily recognized.

Symptoms

As pointed out in the introduction, it is difficult to evaluate clinical alkalosis in terms of specific symptoms in infants suffering alkalosis from continued vomiting. However the constantly occurring postoperative alkalosis, described previously [10], affords an opportunity to observe cases of approximately pure alkalosis in patients usually well hydrated and showing no other gross electrolyte abnormalities. The same applies to the residual alkalosis in patients recovering from respiratory acidosis mentioned above. On repeated occasions, the author has been impressed by the almost complete lack of clinical symptoms in such infants who may feed well, not vomit at all, and appear vigorous and well-being, — even though the pH may have reached 7.55. In most instances, however postoperative alkalosis does not reach severe degrees.

Whereas slight or moderate hypoventilation is very easily overlooked at the clinical examination more pronounced hypoventilation may be indicated by a shallow irregular type of breathing. In several of the patients studied here, hypoventilation was noted on admission, but it was not possible to estimate the level of pCO from the clinical impression. Assessment of the respiratory rate may be possible only by means of the stethoscope but slow respiratory rates may signify hypoventilation.

On admission seven infants (25 %) were described as somewhat hypertonic with stiff movements and occasional twitchings but without the signs of Chvostek and Trousseau. In one patient (Case 1 Table 1) two fits of tonic spasms were observed before pylorotomy was carried out. This patient presented with severe alkalosis, but before the first convulsion occurred the acid base status of the blood had become nearly normal (pH 7.45 base excess +6.1 mEq/l, pCO 45 mm Hg). A review of the clinical records of additional 25 infants with pyloric stenosis, admitted during the period 1959–61 likewise failed to disclose any indisputable instance of latent or manifest gastric tetany.

In the author's experience, the alkalotic infant usually (pre- as well as postoperatively) looks pale, i.e. definitely more so than suggested by the actual hemoglobin value, this pallor tending to disappear during specific treatment.

Treatment

In all cases of severe metabolic alkalosis and whenever pylorotomy was contemplated in the presence of significant alkalosis, a 167 mEq/l solution (0.9 %) of ammonium chloride was administered. Sixteen infants received the ammonium chloride as intravenous infusions. The initial dose was calculated by the formula

$$\text{Dose (mEq)} = 0.3 \times \text{Body Weight (kg)} \times \text{Base Excess} \quad (i)$$

originally developed by Mellemgaard & Astrup [25] to give the amount of bicarbonate required to neutralize the extracellular compartment in metabolic acidosis in adults. The calculated dose usually administered in a scalp vein infusion and often mixed with saline or

fluids containing glucose and potassium was infused continuously in the course of 8–20 hours after which residual alkalosis, as well as additional alkalosis due to continued vomiting was treated by further infusions. The response to this treatment has been uniformly prompt and satisfying (Fig 2, a–d) and by this mode of administration without any side-effects

In the presence of persistent vomiting and gastric retention administration of NH_4Cl by the oral route is obviously inexpedient and usually without significant effect upon the alkalosis (Fig. 2a) Moreover in the patients studied here, oral feedings of NH_4Cl-containing fluids during the pre-operative period often resulted in increasing nausea and more frequent, blood tinged vomitings. Oral administration of NH_4Cl during the immediate post-operative phase should probably be abandoned too

In auto-experiments, the author found the 167 mEq/l solution of ammonium chloride to be moderately irritating following subcutaneous injection while the addition of equal quantities of Ringer's solution diminished this effect considerably Tissue damage following subcutaneous application of NH_4Cl in alkalotic infants was not observed. However where continued vomiting dehydration, and alkalosis call for a safe and fairly short period of pre-operative rehydration and electrolyte therapy the intravenous route is much to prefer Intraperitoneal injection of ammonium chloride was not attempted, as this is reported to cause severe local irritation in dogs [11].

The administration of calculated doses of ammonium chloride in continuous intravenous infusions covering several hours was found to cause no harmful side-effects whatsoever In one case however the intravenous injection of 3.3 mEq of NH_4Cl in the course of 5 minutes in an alkalotic infant (weight *.5 kg base excess +10 0 mEq/l) resulted in sudden collapse greyish pallor and irregular breathing. These symptoms subsided spontaneously within one hour and following pylorotomy the patient recovered uneventfully

Obviously treatment of the alkalosis, as outlined above must be accompanied by correction of the existing losses of water sodium, and potassium (cf [10], and discussion p 140) In the patients described here this was accomplished through the administration of glucose solution (5 5 %), sodium chloride (154 mEq/l) and potassium chloride (61 mEq/l). In severely dehydrated patients KCl, *–3 mEq/kg/24 hours was infused intravenously during the pre-operative period In most cases pylorotomy was undertaken 1–3 days after admission.

Discussion

It is an old clinical experience that infants with pyloric stenosis are particularly predisposed to the development of atelectasis and pneumonia and it is only natural to assume that compensatory hypoventilation in alkalotic patients contributes significantly to the pathogenesis of these pulmonary complications. This would mean that control of the alkalosis is important, especially in patients undergoing pylorotomy Furthermore correction of the alkalosis will facilitate repletion of the body stores of potassium [cf. *1].

Several studies on the use of ammonium chloride in the treatment of metabolic alkalosis are now available [5 8 10,

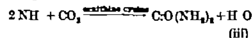
12 14 29 39 42]. In 1943 Zintel and coworkers [4] suggested that parenteral administration of ammonium chloride might be used to combat severe metabolic alkalosis, and a few years later Forbes & Erganian [10], verifying this suggestion, pointed out that treatment with neutral *saline infusions, advocated by earlier* writers [11] with a view to enabling the kidneys to dispose of excess base, can at its best result in slow restoration of neutrality.

In view of the high pK of the ammonium ion (9 1) NH_4 can not be expected to engage directly with the blood buffers, but removal of NH_3 from the blood will cause reaction (ii) to proceed from left to right.



$$NH_4 + \text{buffer base} \rightleftharpoons \text{buffer acid} + NH \quad \text{(ii)}$$

As shown by Fiske & Karsner [9] and later workers, the liver is capable of re*moving large quantities of ammonia from* the perfusing blood. In the liver ammonia is converted to urea, the over-all reaction being



$$2\,NH + CO_2 \xrightarrow{\text{ornithine cycle}} CO(NH_2)_2 + H\,O \quad \text{(iii)}$$

and it is assumed [10] that reaction (iii) is rate-determining for the therapeutic effect of NH_4Cl in metabolic alkalosis.

With splanchnic blood flow [illegible] approximately 20 % of the cardiac output [6] and a cardiac minute volume equal t the blood volume a hepatic clearance (per cent/minut) f ammonia f 20 % would result f NH were removed completely from the blood perfusing the liver By means f the expression

$$S_t = 100(1 - C/100)$$

where S_t is the percentage f NH_4 remaining after t minutes and C the hepatic 'clearance' *f ammonia it can be calculated that for* C = 5 % (implying a constant 25 removal of NH_4^+ from the portal blood) the liver will be able to convert one half f the infused NH in nearly 15 minutes and about 80 % in half an hour In view of the results f Fiske & Karsner [9] and the fact that in early infancy the cardiac output relative t the blood volume is considerably larger than in adults, a 'clearance' of 5 % is probably too low

Thus, the influence of administered NH_4 upon the acid base status as demonstrated in Fig 2 may be explained largely as a result of hepatic trapping of ammonia Because only a small fraction of NH_4 will undergo spontaneous dissociation upon intravenous infusion, and because the Bunsen coefficient of NH_3 for plasma is exceedingly high [16], the resulting pNH will be too low to permit of any significant escape of ammonia through the lungs. NH gaining access to the intracellular compartment reacts with glutamic acid *to form glutamine* [3 20]. *Removal of* ammonia by these means makes possible a quantitative utilization of NH Cl by reaction (ii)

The toxic effects of too rapid intravenous injection of ammonium chloride observed in one patient conform with those reported by Forbes & Erganian [10]. In view of the high pK_A of the ammonium ion, the promptness with which toxic symptoms may be produced [34] and the impressive demonstration by Karr & Hendricks [17] that ammonia toxicity depends upon the rate of injection rather than the dose given and bears no relationship to the resulting pH change, it seems fairly certain that the acute signs of ammonia toxicity must be attributed to toxi qualities of the NH /NH system proper Whereas gaseous ammonia readily

diffuses through the cell membranes the NH_4 ion does not [26], and furthermore it has been shown [41] that the toxicity of ammonia increases rapidly with rising pH of the blood which, according to the laws governing non ionic diffusion [26] facilitates the intracellular accumulation of NH_3. Thus, the administration of ammonium chloride should probably be initiated with the greatest caution when the need for treatment is most urgent, i e in the presence of very high pH values. In view of the needs for simultaneous treatment with potassium, sodium chloride, and glucose, it might be wished to use solutions more concentrated than 167 mEq/l. The author has not found this necessary and the 167 mEq/l solution may be infused with no risk of toxic symptoms.

The formula of Mellemgaard & Astrup [25] has proved a reliable guide to a suitable dosage of ammonium chloride but no case did the initial infusion result in complete restoration of extracellular neutrality [cf Fig .]. Although continued vomiting and the larger extracellular space in infancy partly account for this, the observations presented in Fig 2*b* which include fairly complete urine data suggest that in pyloric obstruction the alkalotic volume is not confined to the extracellular compartment. Assuming a daily endogenous production of non-volatile acid of about 4 mEq (cf. postoperative NAE values in the presence of mild acidosis) the quantity of acid retained (and vomited) by this patient during preoperative therapy amounts to roughly 65 mEq i.e about thrice the amount calculated by formula (I) Actually some vomiting did occur and at the time of operation the patient was slightly acidotic, but still it seems justified to conclude that, prior to therapy a base excess existed inside as well as outside the cells.

Recently some interest has been focused on the relationship of chloride deficiency to metabolic alkalosis [* 21 23]. In view of current concepts of the renal handling of chloride (i.e. passive ionic transfer) it is difficult in terms of the Brønsted theory to account for any *specific* influence of chloride balance upon the acid base status. However if correction of a metabolic alkalosis is to occur without changes in the plasma osmolarity the excess bicarbonate must be replaced by reaction independent anion i e chloride and a lack of renal compensation of metabolic alkalosis in the presence of chloride depletion teleologically equals a protection of normo-osmolarity preferential to restoration of neutrality In terms of tubular processes renal failure to correct metabolic alkalosis in a state of chloride depletion [2] as well as increased excretion (reduced reabsorption) of bicarbonate following intravenous infusion of sodium chloride [30] might be explained by the assumption of a proximal competitive reabsorption of Cl and HCO_3 as thoroughly discussed by Kruhøffer [1]. In pyloric stenosis the concomitant depletion of potassium and sodium is probably equally important in maintaining a positive net acid excretion in spite of prevailing alkalosis [cf 10]

Clinical consequences of the above considerations appear to be that the dangers of severe metabolic alkalosis may be rapidly and safely alleviated by the intravenous infusion of ammonium chloride whereas permanent recovery from alkalosis seems to depend upon rehydration and repletion of body stores of Na K,

and Cl. Because specific clinical symptoms of metabolic alkalosis are few and not easily recognized, the acid base status of the blood should be followed closely during pre and postoperative treatment of hypertrophic pyloric stenosis.

Summary

The diagnostic significance and clinical symptoms of metabolic alkalosis in infancy are discussed on the basis of acid base measurements and clinical observations of 28 infants with hypertrophic pyloric stenosis and a group of patients vomiting from other causes. Experience concerning specific treatment with ammonium chloride is presented, and the rationale of such therapy is discussed in detail.

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The Effect of Long Term Corticosteroid Therapy on Total Red Cell Volume and Haemoglobin Mass in Asthmatic Children

by K. N. AGARWAL and L. E. BRATTEBY

There are some indications that corticosteroids, in addition to their well-known effect on the leucocyte cell system especially lymphocytes and eosinophils, also have an effect on the red-cell system [1]. Fisher [9] reported an increase of total red-cell volume in rats after corticosteroid treatment. Studies of Finch *et al.* [8] have given some evidence of increase in total red-cell volume after corticosteroid therapy in patients with collagenosis.

In a previous study it was observed that after long term therapy with corticosteroids in asthmatic children there was an increase of red-cell count and haemoglobin concentration in the peripheral blood and evidence was presented to indicate that this effect was due to the drug itself [2]. This study presents the results of measurements of total red-cell volume (TRCV) and total haemoglobin (THB) in asthmatic children on long-term corticosteroid therapy.

This investigation was supported by grants from the Swedish Medical Research Council and the International Atomic Energy Agency (Research contract 154/RB).

Fellow on Swedish government scholarship.

The patients were selected from the Sachs Hospital for Children, Stockholm; the Department of Paediatrics, Karolinska Hospital, Stockholm and the Department of Paediatrics, University Hospital, Uppsala.

Adrenaline, aminophylline ephedrine potassium iodide and isoprenaline spray.

Material[1]

Test group: Seven asthmatic children (five boys and two girls) on corticosteroid therapy were selected for the TRCV studies. These children were between 8–14 years old (mean 11 years and 2 months). They were maintained symptom-free (when not under special stress by heavy physical work or infection) on 0.05–0.19 (mean 0.10) mg of prednisone per kg body weight per day for ½–9 years.

Control group: Eighteen children (nine boys and nine girls), maintained symptom-free on drugs[2] other than corticosteroids, were selected as control material. They were in the same age group (6–14 years, mean 11 years). Care was taken to select cases who had not had any infection or any other illness recently. Both in test and control groups, the children had had asthma for at least 3 years. Clinically the subjects in both groups belonged to the same degree of severity (grade I–II) [15] when maintained on respective therapies.

Methods

Haemoglobin determinations were made with the cyanmethaemoglobin method [6].

Haematocrit determinations were made on an International Micro-capillary Centrifuge Model MB. The centrifugation time was 3 minutes. With this technique the volume of trapped plasma has been estimated to be about 1% [10]. In this study no correction has been made for this.

TABLE 1 *Complete data for individual s[illegible]*

Subjects	Age yrs./mth.	Sex	Wt. kg	Ht. cm	Sa. sqm	Hb g %	Hmt %	THB g	TH g/l	TB[illegible] /[illegible]
Control group										
R. W	8 1/12	M	24.5	127.0	0.94	12.4	39.0	161	[illegible]	[illegible]
J. A.	8 2/1	M	21.9	122.8	0.88	12.7	33.5	171	[illegible]	[illegible]
J. G	9 1/12	M	27.5	140.5	1.06	14.3	42.0	225	[illegible]	[illegible]
K. G	9 3/1	M	[illegible]	125.0	0.87	[illegible]	39.0	[illegible]	[illegible]	[illegible]
J. J	9 5/12	M	23.3	131.0	0.95	12.3	35.0	149	[illegible]	[illegible]
E. J	9 9/12	F	24.5	130.5	0.97	[illegible]	40.0	173	[illegible]	[illegible]
S. R.	10 6/12	F	25.5	132.5	0.97	14.5	41.0	223	[illegible]	[illegible]
R. B.	11 1/12	F	30.0	147.0	1.14	12.2	41.0	251	[illegible]	[illegible]
S. F	11 3/12	F	28.5	141.5	1.08	13.0	[illegible]	245	[illegible]	[illegible]
E. H.	11 4/12	F	37.1	152.0	1.29	12.8	37.5	304	[illegible]	[illegible]
S. O	11 9/12	F	35.5	150.0	1.24	13.2	41.0	239	[illegible]	[illegible]
S. B	12 0/12	M	37.2	160.0	1.37	14.9	40.5	303	[illegible]	[illegible]
K. S	12 0/12	F	40.9	164.5	1.26	12.6	[illegible]	326	[illegible]	[illegible]
H. C	1[illegible] 3/12	M	38.1	154.0	1.31	12.0	37.0	342	[illegible]	[illegible]
L. S.	1[illegible] 4/12	M	45.7	[illegible]	1.37	[illegible]	39.0	305	[illegible]	[illegible]
L. E.	12 4/12	F	39.5	155.0	1.33	13.3	40.0	295	[illegible]	[illegible]
A. J	13 10/12	F	36.0	153.0	1.26	[illegible]	40.9	295	[illegible]	[illegible]
L. A.	13 11/12	M	40.0	160.0	1.36	12.3	38.0	304	[illegible]	[illegible]
Test group										
T. L.	8 0/1	M	26.0	112.0	0.90	14.5	43.0	209	[illegible]	[illegible]
L. S	9 4/12	M	22.4	124.5	0.89	12.6	[illegible]	181	[illegible]	[illegible]
A. B	10 0/12	F	22.9	122.0	0.87	14.4	44.0	207	[illegible]	[illegible]
G. A.	12 0/12	M	24.8	134.0	0.97	13.2	41.5	221	[illegible]	[illegible]
E. L.	12 6/12	F	35.5	144.5	1.21	13.5	40.5	290	[illegible]	[illegible]
F. C	13 0/12	M	38.2	142.6	1.23	16.3	48.0	313	[illegible]	[illegible]
J. S.	13 9/1	M	30.3	149.0	1.30	16.1	47.0	339	[illegible]	[illegible]

Sa. (Surface area) is calculated from the formula of Du Bois & Du Bois.

The RCV and THB were determined by a modified Cr^{51} technique to be described in detail later [4]. The activity of the sample was measured in a well-type scintillation crystal. Duration of counting was adjusted so that the statistical error was within ±3%. The amount of Cr^{51} given was 0.04–0.16 μC/kg of body weight (mean 0.08 μC/kg) which gave a total absorbed dose of 4–16 millirads to the critical organ, i.e. the blood. In the calculations of total blood volume (TBV) and total plasma volume (PV), no correction was made for any difference between venous and body haematocrit.

Significance tests were performed according to standard methods [20].

Results

Table 1 presents all the data of both groups. The mean values of haemoglobin and haematocrit in the control group were 13.0 g% and 39.8% respectively. The corresponding values for the test group were 14.4 g% and 43.8 showing a difference of 10.8% and 10% respectively. These differences are statistically highly significant ($P < 0.001$).

In Table 2, values of RCV, THB, TBV and PV per sqm of the body surface area are presented. It is evident that there is a difference of 10.0% in RCV between test and control groups. Similarly the difference with respect to THB is 9.3%. Since the scattering of the individual values in here is comparatively large the observed differences are only possibly significant ($0.05 < P < 0.10$). The calculated PV

...ts in the control and test groups

HB g sqm	RCV ml	RCV ml/kg	RCV ml/sqm	TBV ml	TBV ml/kg	TBV ml/sqm	PV ml	PV ml/kg	PV ml/sqm	Prednisone mg/kg/day
171	482	19.7	512	1263	51.6	1344	781	31.9	831	
199	511	24.1	608	1337	63.1	1502	826	39.0	960	
212	665	24.1	627	1567	57.0	1478	902	32.8	651	
233	631	29.5	725	1617	75.6	1839	986	46.1	1133	
157	422	18.1	444	1161	49.8	1222	739	31.7	778	
178	515	21.0	531	1307	53.3	1347	792	32.3	816	
230	622	24.4	641	1528	59.9	1575	906	35.5	934	
220	745	24.5	654	1860	62.0	1631	1115	37.2	978	
227	759	26.6	703	1846	64.8	1709	1087	38.1	1006	
228	902	24.0	697	2379	64.1	1839	1487	40.1	1152	
192	680	19.4	556	1742	49.9	1405	°053	29.6	849	
230	901	24.0	702	2196	60.0	1729	1305	35.1	1025	
225	963	23.5	706	2325	56.8	1705	1360	33.3	1000	
261	1010	26.5	771	2790	73.2	2130	1780	46.8	1359	
223	945	20.7	690	2443	52.6	1754	1488	31.9	1064	
214	876	22.2	659	2167	54.9	1629	1291	32.7	971	
228	912	23.3	730	2341	65.0	2373	1429	39.7	1143	
224	949	22.7	698	2443	61.3	1804	1504	37.6	1106	
232	620	22.1	689	1443	53.6	1603	823	30.6	914	0.19
207	574	25.6	645	1350	60.3	1517	776	34.6	872	0.12
238	633	27.0	728	1439	62.8	1654	806	35.2	926	0.08
228	666	28.1	718	1677	67.6	1759	951	39.6	1011	0.07
231	841	23.5	896	2075	58.0	1716	1235	34.5	1021	0.05
254	850	24.9	772	1994	57.4	1821	1044	27.3	849	0.06
261	1002	25.1	771	2196	55.1	1891	1191	29.9	916	0.09

in the test group is 6.8 % less than that of the control group. No statistical calculations for plasma volume have been made, as the values are obtained by an indirect method. The mean TBV was identical in the two groups.

Table 3 gives the corresponding values referred to body weight. The observations are in close agreement with those using surface area as a reference (Table)

Discussion

Only a few studies have been made to estimate RCV and THB in childhood [5 14 18 19 1] Furthermore different direct and indirect techniques have been used so that they do not furnish fully comparable data. Only one study performed by Cr^{51}-technique in children has been reported so far [1]. The material comprised mainly surgical cases of various types. On comparing six haematologically normal patients in the age group 6–1. years (R. D M. T., D S., W D D H and D C.) with the control group of our study the values of the RCV in the two groups are in close agreement. There is of course a possibility that the control material does not represent values which are truly normal for the age For the purpose of this study however the important point is that, on analysis, asthmatic children with and without steroid therapy

TABLE 2 *Mean values for RCV THB PV and TBV in relation to surface area*

	n	Mean value	Difference of means Absolute	Difference of means Relative %	SD	SEM	*P*
RCV ml/m²							
Control group	18	648	+[illegible]	+10.6	[illegible]	20.5	0.05 < *P* 0.10
Test group	7	717			46.7	17.5	
THB g/m							
Control group	18	16	+20.0	+[illegible]	27.0	[illegible]	0.05 < *P* < 0.10
Test group	7	[illegible]			17.8	[illegible]	
PV ml/m²							
Control group	18	[illegible]	−66.0	−6.8	—	—	—
Test group	7	930			—	—	
TBV ml/m²							
Control group	18	1647	0	0	—	—	—
Test group	7	1647			—	—	

do not differ in any major respect other than the therapy

As indicated above the literature on RCV and THB in patients under corticosteroid therapy is extremely scanty [7] In a study by Finch *et al* [8] the effect of ACTH and corticosteroid therapy was examined in a group of [illegible]0 patients, mainly adults afflicted with rheumatoid arthritis. Following a daily dose of 100 mg of cortisone in adults and 50 mg in children for 3 weeks, an increase in RCV was observed, but only in those patients who were previously anaemic. It should be noted that the number of initially nonanaemic patients was limited duration of therapy

TABLE 3 *Mean values for RCV THB PV and TBV in relation to body weight*

	n	Mean value	Difference of means Absolute	Difference of means Relative %	SD	SEM	*P*
RCV ml/kg							
Control group	18	23.4	+[illegible]0	+8.6	[illegible]	0.47	0.05 *P* 0.10
Test group	7	25.4			1.86	0.70	
THB g/kg							
Control group	18	7.83	+0.52	+[illegible]7	[illegible]	0.21	0.10 *P* 0.[illegible]
Test group		8.35			0.50	0.19	
PV ml/kg							
Control group	18	36	2.10	−[illegible]	—	—	—
Test group	7	33.1			—	—	
TBV ml/kg							
Control group	18	[illegible]	0.2	−0.5	—	—	—
Test group	7	59.2			—	—	

relatively short and that the RCV was determined by an indirect technique (Evans blue dye). Furthermore no information was given regarding the number of children in their study. It is therefore difficult to compare the results of these two studies.

Corresponding more closely to the results presented in this paper are those of Fisher [9], who found an increase in RCV after administration of corticosteroids in rats, the maximum effect being observed with 2 mg/kg/day of hydrocortisone.

The results of the present study warrant the conclusion that corticosteroid therapy in man increases THB and RCV. This increase is not reflected in total blood volume. The increase in RCV in the test group is apparently compensated by a decrease in PV. This is in agreement with views usually held regarding the mechanism of regulation of the TBV [11, 12, 17, 22].

The difference in RCV and THB between the test group and the control group is about 10 %, and is quite similar to the differences of haemoglobin and haematocrit in the peripheral blood.

Neither the body weight nor the surface area represent an ideal reference for RCV and THB since the variability is larger than when lean body mass is taken as reference [13, 16]. Patients on corticosteroid therapy show increase in body weight mostly of "non-lean body mass" [22]. This increase in weight lowers the calculated RCV and THB with reference to body weight and surface area. Rabbits receiving 1 mg/kg/day of prednisolone showed an increase in peripheral haemoglobin of 6 and an increase in body weight of 6.7 % [3]. This fact reinforces the view that the observed increase in RCV and THB (per sqm and kg) in the test group might have been larger had not therapy caused an increase in weight.

The test group includes only seven cases. Therefore it is not possible to evaluate whether the increase in RCV and THB is dependent upon size of dose or not. It is, however, evident, that some of the patients who showed a higher value for RCV and THB were receiving larger doses than the other patients. They also had clinical features of Cushing's syndrome. A dose-dependent response was evident in a previous study of peripheral blood values under corticosteroid therapy [2].

Summary

Total red-cell volume and total haemoglobin studies were performed by Cr^{51} technique on 25 selected asthmatic children. Seven children were under corticosteroid therapy (test group) and 18 children maintained on drugs other than corticosteroids (control group). Results of this study indicate that total red-cell volume and total haemoglobin mass increase after long term corticosteroid therapy.

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group In six out of 41 patients belonging to the test group, red-cell values are missing These samples, taken on one and the same day could not be analysed due to a temporary dysfunction of the Cellocope The difference in mean haemoglobin values between the two groups is 1.4 g %, which is highly significant ($P<0.001$). Mean values for haematocrit in the two groups differ by 5.3 % and the red-cell counts by 0.48 mill/mm³ and these differences are again highly significant ($P<0.001$) The difference in the means of the two groups for mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are not significant

The haemoglobin values of the test group arranged according to current corticosteroid dosage are presented in Table 5 It is evident that in each age group the mean haemoglobin values are higher than those of normal children (see Table 3) The difference between the groups on less than 0.49 and on 0.50–0.99 mg/kg/day of hydrocortisone is 0.90 g %, this difference being highly significant ($P<0.001$). A similar difference exists between the groups on less than 0.49 and those on 1.00–1.99 mg/kg/day of hydrocortisone ($P<0.001$) On the other hand no *significant difference* [illegible]*xists between* the groups on 0.50–0.99 and 1.00–1.99 mg kg day

Blood examinations were also carried out by th author on some children, who could be taken off corticosteroid therapy for some time Table 6 presents values obtained from [illegible] weeks to 4 months (mean [illegible] months) after discontinuation of therapy The difference between the mean haemoglobin value obtained during corticosteroid therapy of these 13 children and that obtained after discontinuation of therapy is 1.[illegible] g %, which is significant ($0.005<P>0.001$). The difference between the mean haematocrit values in the two groups 5.1 % is highly significant ($P<0.001$) The difference between the mean red-cell counts in the two groups is 0.[illegible] mill mm³ and is possibly significant ($0.100>P>0.050$) The differences in MCV MCH, and MCHC are not statistically significant (Table 6).

In Table [illegible] are given the results of various additional examinations performed on the children belonging to the test group On the whole they do not show any marked deviation from normal, the only exception being the eosinophils which in spite of prolonged corticosteroid therapy show values above normal. It should be noted that the reticulocyte values in this group do not show an increase The bilirubin values do not differ from those obtained with the same method in the control group and the haptoglobin values are also within normal limits.

TABLE 3 *Haemoglobin values of normal children and the control group.*

Age group yrs.	Haemoglobin g Vahlquist (1943)	Guest & Brown (1957)	Control group, present study
		11.5	
1		[illegible]	
2–4	[illegible]	[illegible]	
5–6		[illegible]	[illegible]
8	[illegible]	[illegible]	12.0
9–10		[illegible]	[illegible]
11–14	13.0	[illegible]	[illegible]

TABLE 1 *Distribution of age and sex in the two groups*

Age group yrs.	Test group ♂	♀	Total	Control group ♂	♀	Total
– 2	1	1	2	–	–	–
2– 5	6	0	6	–	–	–
6–10	12	12	24	4	3	7
11–15	8	10	18	7	6	13
Total	27	23	50	11	9	20

values were determined in all patients: haemoglobin, red-cell count and haematocrit. In a selected number of cases in both groups serum bilirubin was also determined. Furthermore, in some of the children belonging to the test group analyses were made also of white cells (total and differential), thrombocytes, reticulocytes and serum haptoglobin.

All these analyses except for haptoglobin estimation were performed by the author himself.

Methods

Haemoglobin: The cyan-methaemoglobin method [5].

Haematocrit: International Micro-capillary centrifuge Model MB 3 min centrifugation.

Red-cell count: Celloscope L. Ljungberg Ltd. Sweden.

Total leucocytes: Burker chamber. Turk's solution.

Differential count: Pappenheimer staining. 200 cells counted.

Reticulocytes: Brilliant cresyl blue staining. 500 cells counted.

Serum bilirubin: According to Michaëlsson [17].

Serum haptoglobin: According to Owen, Better & Hoban [18].

The haptoglobin analyses were performed at the Laboratory of Clinical Chemistry, Uppsala.

Retrospective study

For comparison a tabulation was made also of haemoglobin values from the hospital records of the 50 children belonging to the test group. These are the results of examinations by different technicians and to some extent also by different methods, and should therefore be evaluated with caution. Nevertheless, they deserve some attention, since they represent observations over long periods of time.

In order to make possible a comparison between different individuals irrespective of differences in age a correction has been made in this material for the haemoglobin values according to the following formula,

$$\frac{Hb \cdot 100}{Hbn}$$

Hb = haemoglobin from the case sheet in g %.

Hbn = normal value for age in g % (see Table 3).

The normal values for haemoglobin concentration in children of different ages used in this formula are taken from Guest & Brown [11] and given in Table 3. These values are almost identical with those obtained by Mahlquist [1] in a study of healthy Swedish children of three different age groups and also with those of the control material referred to above.

Results

Actual study

Table 4 gives the blood values and calculated indices of the test and the control

TABLE 2 *Duration of corticosteroid therapy in the test group*

Duration of corticosteroid therapy yrs.	♂		Total
< 1	1	0	1
1–2	7	6	13
2–3	6	2	8
3–5	5	11	16
> 5	8	4	12
Total	27	23	50

TABLE 6 *Blood values during and after discontinuation of corticosteroid therapy*

Investigations	n	Mean	SD	SEM	P
Haemoglobin g %	13				
During therapy		14.4	1.21	0.35	0.003 > P > 0.001
Without therapy		1_.7	1.06	0.31	
Haematocrit %	13				
During therapy		44.5	2.82	0.81	< 0.001
Without therapy		39.4	_.65	0.82	
Red-cell count mill./mm³	11				
During therapy		4.60	0.39	0.12	0.100 > P > 0.050
Without therapy		4.47	0.34	0.10	
MCV cu μ	11				
During therapy		91.8	7.12	_.14	
Without therapy		88.7	6.90	_.04	
MCHC %	11				
During therapy		32.0	1.64	0.49	
Without therapy		31.9	1.65	0.50	
MCH g 10⁻¹²	11				
During therapy		30.0	2.18	0.66	
Without therapy		28.9	2.33	0.70	

Age of the 11 patients 4½–14 years and mean 9 years and 10 months.

year In these mean values have been used

Table 9 presents the haemoglobin values during different periods of the first year of therapy The figures indicate that the maximal rise may already be achieved during the first three months The differences in haemoglobin means for the various dosage levels are not significant.

Discussion

Previous experience including animal experiments and clinical observations, indicate that corticosteroids exert a stimulating effect on erythropoiesis [1 16 19]. So far no studies are available dealing with human beings on long-term corticosteroid therapy who have been free

TABLE 7 *Supplementary values of the test group*

Investigations	n	Median	Range
Total leucocyte count/mm³	14	6000	4600–8700
Neutrophils/mm³	14		
Segmented cells		2750	1672–6794
Stab cells		78	0–237
Eosinophils/mm³	14	690	183–1476
Basophils/mm³	14	70	0–32_
Lymphocytes/mm³	14	2071	1032–4785
Monocytes/mm³	14	547	0–1044
Thrombocytes 1000/mm³	14	270	173–899
Reticulocytes %	_5	1.00	0.50–_.00
Haptoglobin mg %	9	140	90–185
Serum bilirubin mg %			
Control group	10	0.29	0.10–0.50
Test group	14	0.31	0.10–0.54

TABLE 4 *Blood values of the control and test groups*

Mean values for "representative sample of children in age group 10 years (11): MCV = 81 ± 0.6 cu μ, MCHC = 34.3 ± 0.14 %, MCH = 27.8 ± 0.26 g × 10⁻¹²

Investigations	n	Mean	Difference of means	SD	SEM	P*
Haemoglobin g %						
Control group	20	12.9	1.4	0.61	0.12	<0.001
Test group	41	14.3		1.00	0.16	
Haematocrit %						
Control group	40	39.2	5.3	2.10	0.47	0.001
Test group	41	44.5		2.84	0.44	
Red-cell count mill./mm						
Control group	40	4.23	0.48	0.33	0.07	0.001
Test group	35	4.71		0.41	0.08	
MCV cu μ						
Control group	20	92.7	0.40	4.97	1.14	
Test group	35	93.1		4.99	0.84	
MCHC %						
Control group	40	32.7	0.60	1.50	0.34	
Test group	35	32.1		1.85	0.32	
MCH g × 10⁻¹²						
Control group	40	30.7	0.70	1.50	0.33	
Test group	35	30.0		1.80	0.31	

P is the probability that the difference is due to random factors (calculated by student t-test).

Retrospective study

Table 8 shows haemoglobin levels obtained during the year prior to therapy and for each one of the following three years during corticosteroid therapy. It is evident that the rise in haemoglobin appears already during the first year of therapy. Not all of the children had been under corticosteroid therapy for the whole period of 3 years. Furthermore, for part of the material haemoglobin determination was done irregularly and/or with long intervals. These two facts taken together explain why the number of patients in the different groups of Table 8 do not correspond to the total number of the patients in the group under study. In quite a few children, on the other hand, more than one haemoglobin determination, sometimes 3–5, were made during one and the same

TABLE 5 *Haemoglobin values arranged according to current corticosteroid dosage*

Dose of hydrocortisone or hydrocortisone equivalents mg/kg/day

< 0.49				0.50–0.99				1.00–1.99*			
n	Hb g mean	SD	SEM	n	Hb g mean	SD	SEM	n	Hb g mean	SD	SEM
8	12.7	0.53	0.19	19	14.6	0.97	0.22	13	14.5	1.14	0.3

Conversion to hydrocortisone equivalent from other types of corticosteroid according to Di Raimondo & Forsham (6), e.g. 1 mg of prednisolone equivalent to 5 mg of hydrocortisone.
* In two patients the dosage exceeded 2.0 mg/kg/day.

with asthma treated with other drugs ("control group")

The haemoglobin and red-cell values of the control group correspond well with those observed in normal, healthy children [11]. The children in the control group showed an increase in MCV and a dec rease in MCHC, such as has been observed in chronic emphysema.

The children in the test group represen ted more severe cases than those in the control group, but under therapy the situation in both groups with respect to the severity of symptoms was comparable The type of corticosteroid preparation used varied from patient to patient and also with different periods in one and the same patient. In calculating the dosage per kg per day all dosages were transferred to hydrocortisone equivalents according to the table given by Di Raimondo & Forsham [6]. Groups were subdivided with respect to dosage and duration of therapy

From the results presented in Tables 4, 6 and 8 it is obvious that long term steroid therapy regularly gives rise to an increase in haemoglobin and red-cell values. This effect is observed also with a dosage below 0 49 mg hydrocortisone/kg/day (Tables 5 and 9) and often within 3 months of starting therapy (Table 9) An increase in dosage up to the range 1.00–2 49 mg hydrocortisone/kg/day does not give rise to any further increase in the values mentioned (Table 9) In animal experiments it has been shown that corti costeroids administered over weeks nd months cause an elevation of haemoglobin and haematocrit values, but only if the dosage of corticosteroids does not exceed 10 mg hydrocortisone/kg/day With higher dosage the values again decline [7]. The red cell indices in the test group do not differ from those of the control group (i.e. MCV raised and MCHC lowered). In some of the children a follow-up study was performed following cessation of corticosteroid the rapy After a time varying from 2 weeks to 4 months, the haemoglobin and red cell values were again back to normal levels.

The changes in haemoglobin and red cell values do not necessarily reflect the changes taking place with respect to total haemoglobin mass and total red-cell vol ume However in a separate study [ ] this problem has been analysed in some detail and this study has revealed that they do so fairly accurately in this situa tion.

The increase in haemoglobin values was also evident on scrutinizing the hospi tal records and the blood values registered there over the years (Tables 8 and 9). Admittedly such figures must be evalua ted with great caution, nevertheless, it is of interest that the results collected in this way correspond well with the results of the studies by the author referred to above

The rise of haemoglobin and red-cell values in corticosteroid treated asthmatic children could hardly be explained other than as a direct effect of the treatment. An indirect effect by improvement of the asthma situation *per se* could not be the explanation, since an amelioration of oxygen saturation should, if anything induce a decrease in haemoglobin levels.

The explanation of the rise in haemoglobin and red-cell values under corti costeroid therapy is not clear and may be complex. Only glucocorticoids exert su h an effect, and not mineralocorticoids [9].

TABLE 8 *Haemoglobin values obtained from the hospital records*

Group	n	Doses of hydrocortisone mg/kg/day Mean	Range	Hb %	SD	SEM
Prior to therapy	26	–	–	98.7	7 41	1 45
During 1st year of therapy	33	1.56	0.20-2.50	104.4	5 29	0 91
During 2nd year of therapy	28	1.51	0.20–.50	111 1	7.62	1 44
During 3rd year of therapy	30	1.35	0.20–.00	107 7	7.37	1. 3

from basic disorders of the blood and the blood forming organs. In this study the haematological effect of long term corticosteroid therapy has been studied in children with chronic bronchial asthma. The main emphasis is laid on haemoglobin, red-cell values and red-cell indices

Bronchial asthma often gives rise to eosinophilia but does not as a rule affect the haemoglobin and red-cell values In chronic bronchial emphysema, with markedly reduced oxygen saturation the red cell indices show some alterations, mainly an increase in mean corpuscular volume (MCV) and a reduction of mean corpuscular haemoglobin concentration (MCHC) These changes usually balance each other so that the mean haemoglobin content of the erythrocytes (MCH) remains constant [10 12 13 20 23]. In contrast to the situation in reduced oxygen saturation due to high altitude where haemoglobin concentration and even more so total haemoglobin mass is raised, haemoglobin concentration usually remains normal in chronic emphysema [12 16 20]. The explanation for this difference is not clear

In the present study 50 children with asthma treated with corticosteroids ("test group") were compared with 20 children

TABLE 9 *Haemoglobin values for the first year of corticosteroid therapy*

Duration of therapy in months	Doses of hydrocortisone equivalent mg/kg/day < 0.49		0.50–0.99		1.00–.40*		Total			
	n	Hb % mean	n	Hb % mean	n	Hb % mean	n	Hb mean	SD	SEM
Before therapy	–	–	–		–	–	26	98.7	7 41	1 45
0– 3	8	103.7	9	107 4	8	104.5	25	103.9	4.89	0 98
4– 6	4	108.6	11	109.5	3	101 4	18	107.2	7 00	1.61
7 9	2	111.5	7	110.9	4	111 9	13	111 4	9 71	.89
10–1	10	108.7	5	107 7	4	113.5	19	110 0	8 85	01
n	24		32		19					
Mean		108 6		109.6		109.6				
SD		6.34		17.0		11.5				
SEM		1.29		2.99		2.61				–

* Five values were obtained during therapy with dosage exceeding 2.5 mg/kg/day

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Several observations indicate that corticosteroids activate erythropoiesis. *In pure red-cell anaemia and also in other types of aplastic anaemia corticosteroids often induce a response with marked reticulocytosis and rising haemoglobin values.* Correspondingly in animal experiments reticulocytosis and increased Fe^{59} incorporation has been noted under corticosteroid therapy [8]. The *relative increase* in erythropoiesis need not be very large in order to produce the 10 % *increase in haemoglobin concentration and total haemoglobin mass* [2] *observed in our studies*. This may explain why possibly increased erythropoiesis might not reflect itself in raised reticulocyte values. On the other hand it may be that the survival time of the *erythrocytes* is influenced *by* the corticosteroid therapy. *If there is an increased survival time this* may be the whole or partial explanation of the increase in haemoglobin and red-cell values observed. For the time being though data are lacking with respect to red-cell survival under corticosteroid therapy.

Part of the corticosteroid treated asthma material was examined for a number of other data i.e. *leucocyte* counts, thrombocyte counts, smears, reticulocyte counts, *serum* bilirubin, serum haptoglobin. *The only abnormality was an eosinophilia* with eosinophil counts still after years of therapy remaining high, the *median level being* 690 *cells/mm*^{3} as compared with a normal value of some 200 cells/mm^3 [24].

Summary

A group of 50 children with asthma under prolonged corticosteroid therapy showed a significant increase in haemoglobin and haematocrit values. This increase was confirmed in three ways: a comparison between two groups of asthma children, with or without corticosteroid therapy (author's own values); a *follow-up study of part of the corticosteroid treated children* after postponement of therapy (author's own values); and finally a study of the pertinent values in one and the same *group of children, before and during corticosteroid* therapy (values *from hospital* records).

There *was no evidence that corticosteroids will influence the various red-cell* indices (MCV, *MCHC and MCH*).

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the serum bilirubin level under 15 mg/100 ml, were not included in the material. The eleven infant were thus seriously ill and desput repeated blood exchanges the highest serum biblirubin level in two f them was 35 mg/100 ml and 40 mg/100 ml, respectively and in a further six it exceeded 20 mg/100 ml. All had normal birth weight

6. Thirty-eight infants with so-called idiopathic hyperbilirubinemia, where the highest serum bilirubin value varied between 15 mg/100 ml and 20 mg/100 ml. Five in fants were premature by weight (2000 to 2500 g). The others were fullterm.

7 Twenty-nine newborns with idiopathic hyperbilirubinemia, where the highest se rum bilirubin value varied between 20 and 30 mg/100 ml. Two were premature witl birth weight f 1660 and 2240 g respectively The others were mature Three underwent exchange transfusions.

Method

The reaction L-leucyl-β-naphthylamide + $H_2O \rightarrow$ leucine + β-naphthylamine is cata lyzed by aminopeptidase The amount f β-naphthylamine is determined spectrophotometrically after diazotization with dia zotized 2-chlor-4-nitraniline The method has been used according t the process described in Biochemica Boehringer and with ts reagents. It is based upon investigations by Green & co-workers [8] and Braun Falco & co-workers [2].

P du

Two point five ml f a solution f L-leu cyl-β naphthylamidehydrochloride (6 mg/ ml) and 0.05 ml serum are pipetted into 10 ml centrifuge tube Mixed and incubated at 37°C in a waterbath after which .5 ml 6% perchloric acid is added. Mixed well and centrifuged for 5 minutes at 3000 rpm From the supernatant 1 0 ml is pipetted mt a 10 ml centrifuge tube t which is added 1 0 ml diazotized 2-chlor-4-nitraniline (1 mg/ml) Mixed and allowed to stand at room temperature for 10 minutes, after which 5 ml acetic ether is added. The tubes are sealed with cork stoppers, shaken vigorously and centrifuged for period f 3 minutes at 3000 rpm. The red-coloured super natant is put into a cuvett and the extinc tion is read in Beckman B spectrophotometer t 490 mμ against a blank prepared in the same manner but without serum.

With known mounts of β-naphthylamine a standard curve was plotted, and the amount f β-naphthylamine in the serum test was calculated.

The enzyme activity is expressed in units, where 1 leucine aminopeptidase-unit is defined as the amount f enzyme contained in 1 ml serum that releases 1 gamma β-naphthylamine in 1 hour t 37°C.

Serum bilirubin is determined according t a micromethod based upon With modification f the method f Jendrassik & Grof [23].

Results

For the three normal groups: newborn infants where tests were taken from um bilical cord blood, children and non-icteric newborn infants, the mean value the range and the 2 sigma value are shown in Table 1

The results of the determinations in serum from the icteric newborn infants are given in Fig. 1–4, where along the abscissa the age in days and along the ordinate leucine aminopeptidase activity in units are given. From one and the same patient, except in 7 instances two or more determinations have been made. Where the aminopeptidase value has on some occasions exceeded 441 units, the patient s values have been connected by a line in these diagrams.

In T ble 1 it can be seen that the um bilical cord blood and serum from new born infants show somewhat higher aminopeptidase activities than serum from children.

In the following leucine aminopeptidase

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Serum Leucine Aminopeptidase in Icterus of the Newborn

by BENGT KJELLMAN

Leucine aminopeptidase is a proteolytic enzyme that hydrolyzes aminoacid amides dipeptides and polypeptides as well as genuine albumins [20]. The enzyme could be shown to occur in most tissues and body fluids [12]. Increased serum activity is established in intra or extra hepatically conditioned bile stasis [1 10 11 16] and moderately increased values can be demonstrated in liver parenchymal disease [1 7 13 16 22]

Most authors are of the opinion that the clinical value of this enzyme determination is comparable to the determination of ... phosphatases [10 13, 14 15] whereas others find the serum aminopeptidases more often and earlier increased than alkaline phosphatases in cholestatic conditions [1 9 16]

In pregnancy serum leucine aminopeptidase values rise gradually and reach their peak in partus [3 10]. The enzyme activity in umbilical cord blood has been found to be somewhat higher than in serum from adolescents but lower than that of the mother [4 5].

A group of American researchers in 1961 and 1962 presented reports concerning the determination of serum leucine aminopeptidase as a differential diagnosis between neonatal hepatitis and biliary atresia [17 18]. The authors considered that the test was a very sensitive index of cholestasis.

The purpose of the present work was to study the level of leucine aminopeptidase in serum of newborn infants with different type and degree of icterus. As comparison the normal values in children in cord blood and in normal newborn infants were established.

Material

1 Fifty children (age 1 15 years) without biliary or hepatic conditions.

2. Forty normal, newborn infants. The test was taken capillary on the 2nd day of life up to and including the 8th day

3. Blood from the umbilical cord of 44 normal, newborn infants.

4 Eight newborn infants with icterus caused by ABO immunization. The diagnosis was established by demonstrating immune antibodies in the blood of the mother. Highest serum bilirubin value for the 8 infants was 15.5 18.0 18.0 20.0 20.5, 21.0 22.0, 29.0 mg/100 ml, respectively. All 8 had normal birth weight. Exchange transfusion was given in one instance

5. Eleven newborn infants with hemolytic condition caused by Rh immunization. The diagnosis was established by positive Coombs test in the blood of the infant and by demonstrated immune antibody titer in the blood of the mother. The titers varied between 1 16 and 1 1024. Those children where the hemolytic element was slight or where the blood exchange transfusion kept

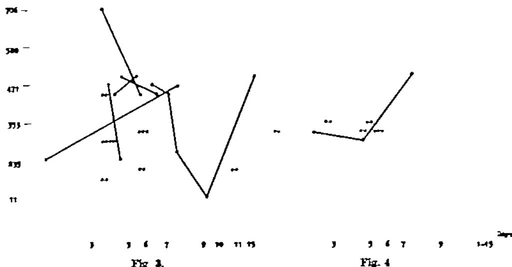


Fig 3. Fig. 4

Fig. 3. Serum leucin aminopeptidase from 29 newborns with idiopathic icterus. Maximal serum bilirubin was > 20 mg/100 ml.

Fig. 4. Serum leucin aminopeptidase from 33 newborns with idiopathic icterus. Maximal serum bilirubin was 15.0–19.99 mg/100 ml

were 4.57 mil./mm³ Serum transaminases were SGO-T 15 units (Karmen) SGP T 10 units (Karmen) Serum leucine aminopeptidase was 206 units. A third blood exchange was made and serum bilirubin was 20 mg/100 ml the following day Reticulocytes 5.5% of 4,5 mil./mm³ red corpuscles. On the fifth day of life serum bilirubin was 11 0 mg/100 ml with negative direct reaction, leucine aminopeptidase 647 units, thymol 0 03. The child p-peared to be well without any neurologic signs deviating from normal. It had normal colored stools. It was sent home at age 6 days.

In the newborn infants group—with idiopathic hyperbilirubinemia and where the highest serum bilirubin did not exceed 19.9 mg/100 ml, one infant had a leucine aminopeptidase value of 500 units and the rest had 441 units or less. The group was composed of 33 infants on whom 66 determinations of serum leucine aminopeptidase was made.

One of the newborns in the group with idiopathic hyperbilirubinemia where the highest serum bilirubin value was 20 mg/100 ml or more had a pathologic ami nopeptidase value (706 units); three infants reached 500 units. The group was composed of 29 infants on whom 60 enzyme determinations were made. The infant with the high leucine aminopeptidase value of 706 units had the following clinical data It was born in normal delivery and had the birth weight of 4180 g. At age 3 days, serum bilirubin was 30 mg/100 ml with negative direct reaction, hemoglobin was 18 0 g/100 ml, and red corpuscles were 4.5 mil./mm³ The following day serum bilirubin was 22 mg/100 ml, hemoglobin was 17.4 g/100 ml. Exchange

value exceeding 500 units is regarded as clearly pathological.

Among the 8 newborns with ABO-immunizing conditions, normal enzyme activity is found in all 22 determinations.

One of the 11 Rh immunized newborn infants showed on the 5th day of life a clearly pathologic leucine aminopeptidase value (647 units) This infant had the following clinical data. The mother belonged to blood group A Rh neg the infant to A Rh pos. The immunization titer was 1:32 The infant was born by normal delivery with a birth weight of 3410 g Immediately after partus it was somewhat listless. In umbilical cord blood bilirubin was 9 9 mg/100 ml with direct reacting bilirubin 0 9 mg/100 ml. Hemoglobin was 13 8 g/100 ml, red blood corpuscles 3 0 mill./mm³ and white blood corpuscles 17 000/mm³ Coombs test was positive Blood exchange transfusion was performed immediately but had to be repeated when, 12 hours later serum bilirubin was *6 mg/100 ml. On the second day of life serum bilirubin was 35 mg/100 ml, hemoglobin was 15.0 g/100 ml red blood corpuscles

TABLE 1 *Serum leucin aminopeptidase from cord blood nonicteric newborns and children. The activity of the enzyme is reported in units the definition of which is stated in text*

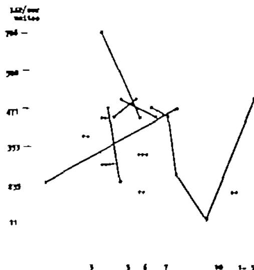
Patients	No.	Mean	Range	2 σ-value
Cord blood	64	293	147–500	198
Normal newborns 2–8 days old	40	263	83–400	194
Children 1 15 years old	50	231	59–471	190



Fig 1 Fig. 2

Fig. 1 Serum leucin aminopeptidase from 8 newborns with ABO-Immunization. Maximal serum bilirubin was 15.5–*9 mg/100 ml.

Fig. 2 Serum leucin aminopeptidase from 11 newborns with Rh-immunization. Maximal serum bilirubin was 14.0–40.0 mg/100 ml.



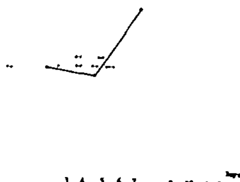


Fig. 3.

Fig. 4.

Fig. 3. Serum leucin aminopeptidase from 29 newborns with idiopathic icterus. Maximal serum bilirubin was > 20 mg/100 ml.

Fig. 4. Serum leucin aminopeptidase from 33 newborns with idiopathic icterus. Maximal serum bilirubin was 15.0–19.99 mg/100 ml

were 4.57 mil./mm³ Serum transaminases were SGO-T 15 units (Karmen) SGP T 10 units (Karmen) Serum leucine aminopeptidase was 206 units. A third blood exchange was made and serum bilirubin was 20 mg/100 ml the following day Reticulocytes 5.5% of 4.5 mil./mm³ red corpuscles. On the fifth day of life serum bilirubin was 11 0 mg/100 ml with negative direct reaction, leucine aminopeptidase 647 units thymol 0 03. The child p-peared to be well without any neurologic signs deviating from normal. It had nor mal colored stools. It was sent home t age 6 days.

In the newborn infants group,—with idiopathic hyperbilirubinemia and where the highest serum bilirubin did not ex ceed 19.9 mg/100 ml, one infant had a leucine aminopeptidase val e of 500 units and the rest had 441 units or less. The group was composed of 38 infants on whom 66 determinations of serum leucine aminopeptidase was made

One of the newborns in the group with idiopathic hyperbilirubinemia where the highest serum bilirubin value was 20 mg/100 ml or more, had a pathologic aminopeptidase value (706 units); three infants reached 500 units. The group was composed of 29 infants on whom 60 enzym determinations were made. The infan with the high leucine aminopeptidas value of 706 units had the following clinica data It was born in normal delivery an had the birth weight of 4180 g. At age days, serum bilirubin was 20 mg/100 ml with negative direct reaction, hemoglobi was 18 0 g/100 ml, and red corpuscle were 4.5 mil./mm³ The following day serum bilirubin was 22 mg/100 ml, hemoglobin was 17 4 g/100 ml. Exchang

transfusion was not made. Leucine aminopeptidase in serum was 706 units at age 4 days and two days later 441 units. The infant appeared consistently well and had normal and colored stools.

Discussion

The leucine aminopeptidase activity is usually expressed in units, but the unit definition varies, different substrates and method modifications and different types of colorimeters have been used. It is therefore often difficult to compare the results of different authors. It is usual to define an enzyme unit as the activity that releases one gamma naphthylamine from the substrate during 2 hours incubation at 37 C. In the present work, one hour's incubation was used, but because the rate of the enzyme process during the first hours is calculated to be constant [8, 13] the doubled values of the author are comparable. Goggel and co-workers [9] have for normal adults a mean value of 483 units/ml with the upper limit of 660 units for men and 740 for women. Gohr [7] indicates the upper normal limit as 750 units/ml. Nikkilä [15] also finds similar normal values, but 3 of the 28 cases investigated by the latter have the values about 1000 units/ml.

The mean values given in Table I of the present work correspond to those of the mentioned authors and to those given by Müllner [14]. A tendency to higher normal values in tests from umbilical cord and from normal newborns than in tests from children can be traced in the author's results, which also corresponds to results of other researchers.

Pineda & co-workers [1] maintain that at "physiologic" icterus or hemolytic conditions in the newborn infant, the leucine aminopeptidase activity in serum should be normal. Two of the 80 newborn infants with hyperbilirubinemia investigated in the present work had pathological serum values. The increase of the enzyme was clear but not up to the very high level, which is reported to exist in biliary atresia or sometimes in different conditions of choledochus obstruction [18].

One of these two patients was included in the group idiopathic hyperbilirubinemia and had the highest serum bilirubin value in its group (30 mg/100 ml) and the other belonging to the Rh hemolytic diseased infants had the next highest serum bilirubin value (35 mg/100 ml) among these.

At hyperbilirubinemia conditions in newborn infants so-called inspissated bile syndrome is sometimes found as a complication and at Rh hemolytic disease frequency figures of 8–10 have been stated [6, 21].

Whether the increased serum aminopeptidase values in the two patients must be interpreted as expressions for an early subclinical cholestatic condition of similar type as inspissated bile syndrome is unclear. Otherwise the infants had no signs of cholestasis. They had thus consistently normal and colored stools and negative direct reaction at bilirubin determination. Also after discharge from hospital they showed no complications.

Summary

The normal values for serum leucine aminopeptidase, using the method of Green et al. and Braun Falco & Salfeld, have been established in normal newborn

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Studies on Erythro-Kinetics in Infancy

V Estimations of the Life Span of Red Cells in the Newborn

by LARS GARBY, STIG SJÖLIN and JEAN-CLAUDE VUILLE

The life span of normal red cells in adults is well known, but the life span of the red cells formed during foetal life and during the new born period is still a matter of considerable dispute since published estimates are both approximate and conflicting [1, 2, 6, 7, 9, 10, 12, 13, 15]. The present communication deals with an analysis of data obtained in previous studies [4, 5] concerning the kinetic behaviour of radio-iron injected during the neonatal period. The estimates obtained on the basis of these data, although approximate, indicate that the life span of red cells formed during foetal life and during the neonatal period is significantly shorter than that of adult normal red cells.

The life span of red cells formed during the foetal period

In a previous publication [4], data were presented concerning the relative rate of synthesis of HbF and HbA during the first 20 weeks after birth. As will be shown below, these data provide a basis for an estimate of the life span of red cells formed during the latter part of intra uterine life

This investigation was supported by grants from the Swedish Medical Research Council and by grants (Research Project 184/RB) from the International Atomic Energy Agency, Vienna.

Let S^F and S^A be the rates of synthesis of HbF and HbA at any time t, and let D^F and D^A be the rates of destruction of the two haemoglobins at the same time. If M^F and M^A are the amounts of HbF and HbA circulating in the blood at time t

$$S^F - D^F = \frac{dM^F}{dt} \quad (1\text{ a})$$

and

$$S^A - D^A = \frac{dM^A}{dt} \quad (1\text{ b})$$

Let $S^F/S^A = R$ and substitute M^F for $C^F \cdot V$ and M^A for $C^A \cdot V$ where C^F and C^A are the concentrations of HbF and HbA respectively, and V is the blood volume. On dividing equations (1) we have

$$R = \frac{\dfrac{dC^F V}{dt} + D^F}{\dfrac{dC^A V}{dt} + D^A} \quad (2)$$

Destruction coefficients q^F and q^A are defined thus,

$$q^F = D^F/M^F = D^F/C^F \cdot V \quad (3\text{ a})$$

$$q^A = D^A/M^A = D^A/C^A \cdot V \quad (3\text{ b})$$

By substitution,

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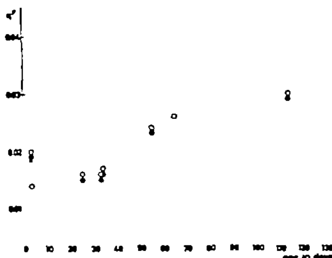


Fig. 1 The destruction coefficient q^F plotted as a function of age. Each infant is represented by three points, ● ○ and corresponding to assumptions of the magnitude of the destruction coefficient q^A of 0.00, 0.004, and 0.02 respectively

of q^F Since the relation (5) contains two unknowns, both values of q^F and q^A will remain unknown unless a value for one or the other can be given in advance. Now since the two quantities are related, as shown in eq. (5) (i.e. an increase in q^A must give a corresponding increase in q^F), it is obvious that a minimum estimate of q^F is obtained by simply letting $q^A = 0$ Larger values of q^A will only give larger values for q^F Fig 1 shows clearly that q^F increases with increasing age This fact is understandable since the fraction of HbF containing cells reaching the age of death must increase with the infant's age and indeed become infinitely large towards the time when the last produced HbF-containing cell dies. It can also be seen from Fig 1 that the estimated value of q^F during the first month of life is between 0.01 and 0.025 depending on the value assigned to q^A Since the value of q^A cannot be accurately estimated, the value of q^F remains unknown within the given limits but it seems difficult to escape the conclusion that it must exceed 0.01 per day Since at the time of birth, the population is skew with a preponderance of young cells the above estimate of q^F is a minimum estimate of the q^F value corresponding to the hypothetical steady state Thus, the mean cell life span of the HbF-containing cells present at birth and formed during the latter part of intrauterine life must be shorter than 1/0.01 = 83 days

The life span of red cells formed during the neonatal period

In a previous publication [5] data were presented concerning the long term behaviour in circulating foetal and adult haemoglobin and in the faeces of radioiron injected intravenously It was found that the amount of radioactivity in circulating HbF usually showed definite signs of falling starting at about 60–90 days after injection. Also there were definite indica-

$$R = \frac{\dfrac{dC^F V}{dt} + q^F C^F V}{\dfrac{dC^A V}{dt} + q^A C^A V} \quad (4)$$

and

$$q^F = \left[R\left(\frac{dC^A V}{dt} + q^A C^A V\right) - \frac{dC^F V}{dt}\right] / C^F V \quad (5)$$

Values of R at different ages from 0 to 20 weeks of life were obtained by Garby, Sjölin & Vuille [4]. At the same time values for C^F and C^A were obtained, so that there remain only three unknown quantities in eq. (5). The blood volume V, however, can be roughly predicted in each individual from the data of Mollison, Veall & Cutbush [8], and of Sisson et al. [14]. It is a specific property of eq. (5) that systematic errors in these predicted blood volumes will have only a slight effect on the values of q^F and q^A. We are thus left with a relation containing two unknowns. Some interesting properties of the two unknowns, q^F and q^A, will now be considered and it will be shown that these properties can be used to reveal some non trivial facts about the unknowns and also about the life span of the red cell formed during the latter part of intrauterine life.

Equations (3) are exactly valid at any one time, but since the absolute destruction rates D^F and D^A are unknown functions of time, no general physiological significance can be attached to the quantities q^F and q^A. However, in one specific situation, the quantity q has a specific physiological significance, namely in the steady state, where $S = D$ and $dM/dt = 0$. In this situation

$$D = M/T \quad (6)$$

where T is the mean cell life span of the red cells. From (3) and (6) it follows that

$$q = 1/T \quad (7)$$

Thus, the q's are equal to the reciprocals of the mean cell life span of the red cells in the steady state. In non-steady states, such as we are dealing with at this period of life, the age distribution of the circulating red cells is constantly changing. The number of cells reaching the expected age of death is therefore also constantly changing, although there may be a constant and definite life span for each red cell. As a corollary, the destruction coefficients q will also change with respect to time, and if the age distribution is skew because of the non steady state, the reciprocal of q will not be identical to the mean cell life span. For instance, since the amount of circulating HbA increases during the period before birth, it can be safely assumed that the age distribution of this haemoglobin at birth is such that its mean age is much less than it would have been in the corresponding steady state. The same argument is valid also in the case of the circulating HbF, although the age distribution of this type of molecule at birth is presumably somewhat less skew. In fact, simple calculations on the age distribution of the red cells at birth on the basis of the total haemoglobin content of the foetus during the months before birth show that, regardless of the approximate nature of the data involved, the age distribution will always be skew with a dominance of young cells. Thus at the time of birth the values for both q^A and q^F are in all probability smaller than the same values obtained under the corresponding hypothetical steady state. An estimate of T (T^A and/or T^F) on the basis of q-values during this period will thus always be an overestimate.

The data obtained on the values of R, C^A, C^F and V at different ages after birth [4] have been used to calculate the value

downward slope of the curve and by plotting the values of the differentials as a function of time Since however there is a considerable time-lag in the incorporation of radioiron into the cells (of the order of 15–20 days, cf Fig 2) the downward slope of the curve is too slight to give correct values by the method indicated above Also, since it has been shown [4] that some synthesis of HbF occurs during this age period, some reutilization of radioiron into HbF must have taken place This phenomenon will also tend to lessen the slope of the downward part of the curve. Since the extent of re-utilization is not known, the appropriate correction for this phenomenon cannot be made. However even if it is assumed that no re-utilization at all takes place the data show that some 30 % of all the HbF-containing cells survive only for 60–100 days. Thus, it seems that quite a considerable fraction of the cells formed during this period of life survive only for some 60–100 days.

The data on th faecal excretion of radioiron are in close accordance with this conclusion. The peak of excretion starts about 0 days after injection, and reaches a maximum at about 90–100 days. In order to interpret the timing of the excretion data in terms of the life span of the labelled cells, the excretion data must be corrected for the three phenomena that tend to delay the ppearance of the radio iron in the faeces. In th first place there is delay caused by emptying of the bowel, this is probably only of the order of one day however In the second place there is delay due to chemical processing of the haemoglobin to yield iron, the data presented by Garby & Noyes [3] on the f t of injected haemoglobin labelled with iron and the findings of Noyes, Bothwell & Finch [11] on the fate of damaged red cells labelled with iron indicate that this time-lag is of the order of a few days. Finally since there is delay in the labelling of the cells (cf. above) all values after the start of the peak should be corrected for this delay the "true" excretion peak must therefore be narrower than that found experimentally It thus seems clear that, to judge from the excretion data, the bulk of the cells formed during the neonatal period survive only for some 65–100 days, a conclusion tallying with the one reached above

Discussion

The analysis of the data given in this paper supports the idea that the mean cell life span of the red cells formed during foetal life and during the neonatal period is shorter than 120 days (which is the mean life span of red cells in adults). This conclusion is in agreement with that of some previous investigators, but is at variance with the conclusion reached by other workers

Mollison [9] transfused placental and adult red cells simultaneously to newborn infants, and followed the fate f the transfused cells by differential agglutination. The results showed that the placental cells disappeared at a somewhat faster rat than the cells from the adult donor The conclusion that the mean life span of the placental cells must be shorter than that f adult red cells is reinforced when it is borne i mind that the placental red-cell population contains a greater proportion of young cells.

Seeleman [13] transfused placental red cells t four infants aged 11 days and 6, 8, and 10 weeks respectively and found by the method f differential agglutination that the transfused cells disappeared by a constant fraction of roughly 0 9 % per day The disppearance rate varied considerably and no

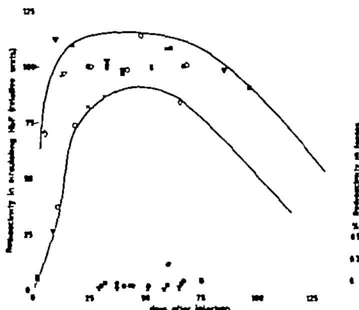


Fig. 2. The time course of the relative amount of radioiron in HbF and in faeces in infants after intravenous injection of radioiron during the neonatal period. Radioactivity in circulating HbF: Case Li, ○ Case Bl, ○ Case Sj, + Case Lö and Case Ja. Radioactivity in faeces: Case Bl, ● Case Sj and Case Ja.

tions that the amount of radioiron found in the faeces increased at about the same time. The data obtained in the five cases of that study (Li, Bl, Sj, Lö and Ja) are represented in a slightly different manner in Fig. 2. The amount of radioactivity present in circulating HbF in each case has here been normalized in order to facilitate a graphical comparison, each value for the percentage of the injected dose in circulating HbF was multiplied by a factor to make the relatively horizontal part of the curves between the *0th and 60th day coincide. The excretion of radioactivity in the three cases studied (Bl, Sj and Ja) was calculated as the percentage of the non-haemoglobin radioactivity in the body.

The data on the relative amount of radioactivity in HbF shown in Fig. 2 indicate very strongly that at least some of the HbF containing cells formed during the first weeks after birth have a life span that is shorter than that of normal red cells of adults, i.e. shorter than 1*0 days. In fact, some of them must have a life span of the order of 60–70 days only. The mean cell life span of the population of cells formed during this period of life cannot be evaluated with any precision, but the following considerations indicate very strongly that the mean cell life must be considerably shorter than 1*0 days. If there were immediate and complete incorporation of the radioiron into the red cell at the time of injection and if there were no re-utilization of radioiron in HbF, then the mean cell life span and the distribution of individual life spans about this mean value could be obtained by differentiation of the

hypothesis that the mean life span of these cells is shorter than the mean life span of adult red cells.

It is pointed out that if some of the earlier work is reinterpreted, all data so far published are in favour of the hypothesis that the red cell life span is shorter during this period of life

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corrections were made for possible changes in blood volume Seeleman interpreted his results as indicating that the life span of the placental cells was 111 days, i.e the reciprocal of the daily fractional disappearance but failed to note that this mode of calculation requires that the age distribution of the transfused cells be uniform. His results must probably be taken to signify that the mean cell life span of the placental cells is shorter than 111 days.

Vest [15] used the same technique in four premature infants, and found that the a er age disappearance fraction was in all cases greater than 0 9% per day If it is assumed that the blood olume in these infants did not change appreciably and bearing in mind the non uniformity of the transfused popula tion, it must be concluded that Vest s results also indicate that the mean cell life span of the placental cells is shorter than that of adult red cells.

Several investigators (Hollingworth [6], Vest [15], Foconi & Sjölin [ ] and Kaplan & Ku Shin Hsu [7]) have labelled cord blood cells with radioactive chromium, and traced the disappearance of chromium activity in such cells after transfusion. If it is assumed that the "elution of chromium *in vivo* is he same in adult and placental red cells ese studies indicat that the mean cell life span of placental cells is shorter than that of adult cells.

Schulman & Smith [1] estimated the total circulating number of red cells in pre mature infants during the first weeks of life from the plasma volume as measured by dye dilution. The decrease in the number of total circulating red cells during the first 5–6 weeks was between 1 1 and 1 3 per day in four premature infants. The reciprocal of thi value i.e 77 93 days is a maximum estimate of the mean cell lif span in these infants, since it is calculated by assuming that no haemoglobin synthes takes place during the period, and by assuming a un form ge distribution of the cells.

Daneis, *et al.* [1] followed the fat f N[1] administered orally as ammon um chloride or glycine in circulating haemoglobin in three premature infants. They interpreted the data as showing that the l fe span of the red cells formed in these infants was nor mal" i.e about 120 days. An inspection of their findings however would seem t show that in two infants, ROM and ROD, the decrease in total circulating haemoglobin \ already started at about 70-90th day fter the administration of the label. Corrections for the delay in labelling and for re-utiliza tion of the labelled nitrogen will reinforce the conclusion that the mean cell life span of the labelled cells is in fact considerably shorter than 1*0 days, unless a quit remark ble spread in individual lif span is assumed. In the third infant TOR, an acut episode of haemolysis during the second month of life made any interpretation impossible

Summing up the results of previous investigators it thus seems that all data so far collected favour the hypothesis that the mean cell life span of the red cells in the newborn whether these cells are formed before or after birth is shorter and possibly considerably shorter than 1*0 d ys This conclusion is in good agreement with the findings now presented.

Whether the shorter mean cell life span during this age period is a result of a shorter potential life span or whether it is a result of external, more or less random, influen ces cannot be said at present although the data of Mollison [9] rather favour the former view

Summary

Data obtained in previou tudies concerning the behaviour of radioiron in circu lating foetal adult haemoglobin and the pattern of excretion of radioiron are ana lysed in term of the life span of red cells formed during foetal life and the neonatal period

The result are compatible only with the



Fig. 1. The patient at 11 days of age.

There is no history f illness, drug ingestion, exposure to X-ray or known toxins during the pregnancy She did receive an injection f poliomyelitis vaccine during the first month. The mother has no past history of serious illnesses.

Cl nical findings

The infant boy was del vered three weeks prematurely by Cesarean section performed because of maternal bleeding which began the previous day His birth weight was 3200 g and his birth length was 50 cm. H was cyanotic and in respiratory distress immedi ately fter delivery but there was improvement fter oxygen administration. It was noted that the umbilical cord contained only one umbilical artery Because of an omphalocele and anal atresia the child was immediately transferred to the Pediatric Surgery Depart ment, Karolinska Sjukhuset, Stockholm. H had a marked micrognathia with macroglossia and in an attempt to improve the airway the tongue was sutured before the transfer for surgery

The child had surgical repair of the omphalocele and anal atresia on the day f birth. The omphalocele was described as being egg-sized and the pedicle was 1.5 cm in diameter A portion of the small intestine was inside the omphalocele and it was found t be incarcerated and discoloured. Only one umbilical artery the right was found. A marked diastasis recti was observed. The anal tresia was of the membranous type. There was thread-like perineal fistula from which meconium wa obtained after probing. The external genitalia were normal.

Post-operati ely the patient's general condition was poor and he continued to have respiratory distress with subcostal and sternal retractions but with no cyanosis. The thoracic cage was soft

At 11 days f ge the child (Fig. 1) was transferred from the Surgery Department of the Pediatric Clinic t the Medical Depart ment. Physical examination revealed the following significant features in addition to those already described:

Scaphocephaly with premature synostosis of the sagittal suture; anterior fontanel (3 3 cm) with normal tension; pronounced micrognathia, a high-arched palat macroglossia; slight hypertelorism; very small palpebral openings; bsence f epicanthic folds; and low-set malformed ears with folding-over f the upper border f the helix so that the upper margin formed a right angle with the descending part of the helix. There was mild scoliosis in the lumbar region. The thoracic cage was soft with respiratory retractions, but auscult tory findings of the chest were normal. There was no hepato-splenomegaly The fingers and toes were unusually long and there was a distinct gap between the first and second toes. There was no simian line



Fig. 2. Pneumoencephalogram showing marked dilatation of the ventricular system.

Neurological examination showed generalized hypotonia with hypoactive Moro reflex and deep tendon reflexes. The stepping and walking reflexes were low.

Ophthalmological examination revealed blepharophimosis and colobomata of the irides on the nasal side in the lower half of each eye. The eye grounds were normal centrally but were not visualized peripherally.

The head circumference, which measured 37 cm at birth, increased to 46 cm at two months of age and at the time of death, when the patient was 3½ months old, the head circumference measured 50 cm.

A bone marrow aspirat was obtained after immature, atypical, vacuolated mononuclear cells were noticed in the peripheral blood. The marrow contained a large number of vacuolated mononuclear cells but was otherwise normal.

An X ray of the skull and a pneumoencephalogram showed synostosis of the sagittal suture and marked dilatation of the ventricular system (Fig. 2). Other radiographic examination showed the presence of a hiatus hernia, malrotation of the small intestine and dilatation of the left renal pelvis. The results of special investigations are summarized in Table 1.

At the age of two months, the infant (weighing 3150 g) was transferred from the Pediatric Clinic, Karolinska Sjukhuset, to a

TABLE 1 *Summary of special investigations of the patient*

Examination	Age of patient (days)	Results
X-ray		
Skull	12	Skull abnormally long and narrow. Premature synostosis of sagittal suture. Other sutures abnormally wide. Fontanelle open.
Pneumoencephalography	27	Marked dilatation of ventricular and cisternal system. Impression: marked central atrophy of the brain (Fig. 2).
Chest	13	Thorax slightly deformed with an increase in the AP diameter. Lungs and heart shadow normal. Diaphragm low.
Spine	16	No anomalies of vertebrae. Slight scoliosis in the lumbar region.
Pelvis	16	Iliac index normal, 80.
Barium swallow esophagus	16	Esophagus curving which is explained by a gliding hiatus hernia.
Cineroentgen of mouth and pharynx during swallowing	17	Smaller than normal space between the root of the tongue and the posterior wall of the pharynx.
GI series	20	Malrotation of small intestine. Hiatus hernia.
Barium enema	18	Anus normal at rest and during defecation.
IVP	16	Normal excretion on left side. Moderate dilatation of left pelvis. Left ureter normal. Right renal pelvis not visualized.
Urethrocystography	17	Reflux during micturition to right ureter and right pelvis. Slight dilatation of right ureter and pelvis. No reflux on the left side. A dilatated utricle in proximal urethra was demonstrated.
EEG	21	Normal.
Serum creatinine		1.2 mg %.
NPN		37 mg %.
Spinal fluid		Normal.
Hgb		15.9 g %.
WBC		Varying between 15,000 and 42,000.
Platelets		Varying between 480,000 and 890,000.
Urinary sediment		Normal.
Urinary proteins		Slightly positive.
Phenylpyruvic acid		Not found in urine

local hospital where the child's general condition remained poor until he died at 3½ months of age.

Cytological studies

The analysis of the patient's chromosomes was based on the study of cell cultures derived from one bone marrow and two skin biopsies. Four primary cell cultures and their subcultures were examined from each biopsy material and the technique developed in this laboratory [5] was used throughout. A total of 102 undamaged cells in mitotic metaphase were counted, including 76 from skin and 26 from bone marrow. Fourteen metaphase plates from skin and one from bone marrow were studied in detail by enlarged photomicrographs. In each instance the karyotype contained only four small acrocentrics and one chromosome which could not be matched. This odd chromosome was submetacentric and had about the same length as chromosome 18. The long arm appeared to be the same length as the short arm of number 4 or 5, and the short arm had about the same length as the long arm of number 21 or 22.

In most of the plates one of the small acrocentric chromosomes was identified as a

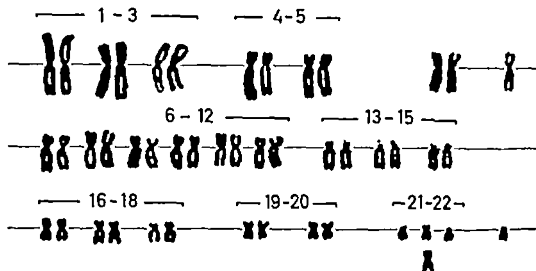


Fig. 3. Karyotype of the index case. There are only three chromosomes in the 21–22 group and one unpaired chromosome (placed below group 21–22) with submedian centromere.

Y chromosome because of the morphological features of straight chromatids which were more parallel than in the other three acrocentrics and the presence of a terminal centromere. The number of satellited small chromosomes never exceeded three in any of the preparations. The karotype is shown in Fig. 3. Cells derived from skin and bone marrow by culture were negative for sex chromatin.

Chromosome studies on the parents were carried out on cultures derived from skin biopsies. Analysis of the cells from the father revealed a normal male karyotype. Analysis of 51 undamaged cells derived from the mother showed a chromosome number of 46. Sixteen metaphases were studied in detail by enlarged photomicrographs. There were only three small acrocentric chromosomes. In addition to the same unusual chromosome present in the karyotype of her son, one of the chromosomes in group 4–5 lacked the short arm (Fig. 4). Typical sex chromatin was found in 45 % of the interphase nuclei of skin. The results of the chromosome analyses are summarized in Table [illegible].

The most likely interpretation of the chromosome abnormality in the mother is a translocation involving a chromosome of group 4–5 and a member of group 21–22. The translocation chromosome with submedian centromere was transmitted to her son, who probably was effectively trisomic for the short arm of a chromosome 4 or 5.

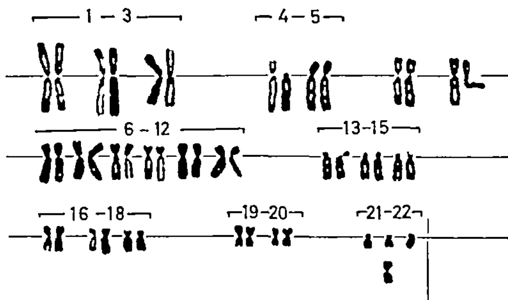


Fig. 4. Karotype of the mother showing lack of the short arm of member of group 4–5, bsence of member of group 21 22, and presence of unpaired chromosome similar to that found in the index case.

Discussion

The translocation found in the present family is probably reciprocal Theoreti cally an individual heterozygous for a reciprocal translocation can produce many kinds of gametes. The behaviour of reciprocal translocations in plants [8] and in the mouse [7] indicates that the gametic out put consists mainly of four types. In the family under discussion these four types of gametes prod ced by the mother and the corresponding zygotes resulting after fertilization with a normal sperm are illustrated in Fig. 5. The first type of gamete shows the translocation chromosome and a normal chromosome no 4 or 5 The zygote formed by the union of this gamete and a normal male gamete has the same chromosome constitution as the index patient, who is effectively trisomic for most of the short arm of no 4 or 5 The second type of gamete is normal. The third type of gam ete contains a chromosome no. 4 or 5 with loss of most of the short arm and the

TABLE 2 *Summary of chromosome analyses of the patient and his parents.*

	Chromosome counts 45	46	47	48	Karyotype interpretation
Patient					
Skin	1	74	3	0	4–5/21–22 translocation
Bon marrow	1	24	1	0	4–5/21 22 translocation
Mother					
Skin		40			4–5/21 22 translocation Lack of the short arm of a hromosome (4–5)
Father					
Skin		15			Normal karyotype

translocation chromosome The zygote formed by the union of this gamete and a normal male gamete would have the same karotype as the mother and would probably develop into a phenotypically normal individual This individual would however again produce gametes of the same four types. The fourth type of gamete shows a chromosome no 4 or 5 with loss of most of its short arm and a normal chromosome 21 The corresponding zygote resulting from the union with a normal male gamete would be grossly deficient and perhaps inviable

Some cases other than mongols, with multiple congenital malformations associated with translocations have been reported Edwards *et al* [9] described two families in each of which the father was presumed to have an autosomal reciprocal translocation as a result of which both children in one family and one of three children in the other showed mental retardation and multiple malformations. The translocatrons were probably between chromosomes nos. 9 and 4 in the first family and chromosomes nos 1 and 6 in the second family A case of 18-trisomy resulting from a translocation of most of the long arm of a no 18 to a chromosome in the (13–15) group was reported by Brodie & Dallaire [4] The translocation was also present in the clinically normal mother Another case with the clinical signs of trisomy 18 syndrome due to a translocation was recently reported [16].

A mother who had had one abortion and three other children who died in early infancy was found to be a carrier of a translocation between a small acrocentric from the group (21–22) and a chromosome of group (17–18) [19]

It is possible that, in the family under discussion, the two embryos lost by miscarriages were chromosomally unbalanced. Some mothers who are heterozygous for a 13–15/21 translocation and have produced children with mongolism carrying this translocation have been found to have a high frequency of miscarriages [13 *0, 23].

Abnormalities in growth and development of about the same severity as found

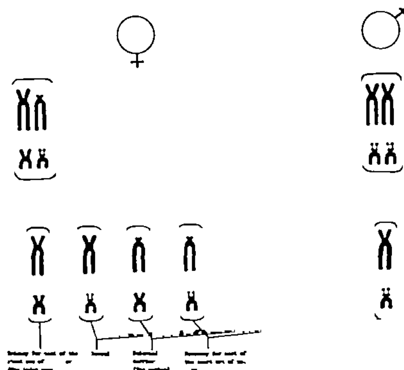


Fig. 5. Schematic drawing showing the four main types of zygotes resulting from the mating between normal father and mother who is carrier of the translocation chromosome.

in this patient are also seen in the (13–15)-trisomy syndrome and the 18-trisomy syndrome.

None of the three established autosomal syndromes, that is, 21 trisomy (13–15)-trisomy or 18-trisomy have the malformation syndrome of this case although some of the features of th present case are a common finding in them. Muscular hypotonia low-set malformed ears coloboma ta of the irides and small palpebral openings are common findings in the (13–15)-trisomy syndrome and micrognathia a high-arched palate low set malformed ears renal anomaly and unusually long fingers in the 18-trisomy syndrome Muscular hypotonia, macroglossia, a high-arched palate ears with an gular folded upper helix and a distinct gap between the first and second toes which are common findings in mongolism were lso found in this patient

The finding in the present case of a missing umbilical artery is interesting because of the association between absence of one umbilical artery and malformations [2, 11].

The abnormalities in the cases with absent umbilical artery are mostly mul tiple and not confined to any particular system but obstructive lesions or tresia of the gastrointestinal and urinary tracts are common. There is no definite indication of the cause of the absence of the umbilical artery or of its exact role in the frequent association with malformations. Absence

of one umbilical artery has been found in cases of 18 trisomy [14, 18] in (13–15)-trisomy [11] and in mongolism [Gustavson, unpublished data].

Summary

Cytogenetical and clinical observations of a child with multiple congenital malformations are reported. Some of the features present are found regularly in patients with established autosomal syndromes, but many others present in this patient are not common to them, i.e. marked hydrocephalus, malrotation of the small intestines, absence of one umbilical artery, omphalocele and anal atresia.

Chromosome studies showed a chromosome number of 46 and an XY sex chromosome constitution. There were only three chromosomes in group (21–22) and there was an odd small submetacentric chromosome which could not be paired. The mother, who was phenotypically normal, had a chromosome number 46. An odd small submetacentric chromosome similar to that found in the karyotype of her son was present and in addition, one of the chromosomes in group (4–5) lacked the short arm. The paternal karyotype was normal. The most likely interpretation of the chromosome abnormality in the mother is a reciprocal translocation involving a chromosome in group (4–5) and a member of group (21–22). This translocation chromosome was transmitted to her son who was effectively trisomic for the short arms of a member of group (4–5).

Note added in proof. Further studies have revealed the maternal grandfather of the propositus to be carrier of the same translocation found [illegible] the cells of his daughter and grandson.

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CASE REPORT

Choroid Plexus Papilloma and Infantile Hydrocephalus

by IRÉNE SJÖGREN GUNNAR GROTTE and LARS OLDING

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Choroid plexus papilloma were des cribed for the first time in 183* by Guérard in Paris. Up to now a total of 160 cases has been described [1 25]. Among Cushing s °000 cases of verified intracranial tumors [5] 13 were cases of choroid plexus papilloma giving a frequency of 0 6 %. Other investigations of their frequency give similar information [1 12, 25]. Choroid plexus papilloma occur more frequently in women than in men [1] In children they are more common than in grown ups [19 24 25] and occur with a frequency of 3.9 % of all intracranial tumors [4]. In 4 cases choroid plexus papilloma have been described in newborns [2 7 13 17].

In a Swedish material of 25 cases [1] of choroid plexus papilloma 15 were localized in the fourth ventricle 2 in the third and 8 in the lateral ventricles, usually in the *left [19 *4]. In children they are* generally localized in the lateral ventricles. One case with tumors in both lateral ventricles at the same time has been reported [20]. Metastatic seeding has been observed in 9 cases out of 86 [19].

The symptoms are generally non specific with headache dizziness, nausea and vomiting. Depending upon the localization and size of the tumor paresis of the eye muscles, protrusion of the papilla, impairment of vision, ataxia and tinnitus may occur In half the cases hydrocephalus occurs, and this is practically always the case in children [19]

The cerebrospinal fluid is usually xanthochromous and shows a rise in protein content (100–500 mg %) [1 13, 17 18, 23, 25], although low total protein contents may also occur [9 20].

X ray investigations often show enlargement of the skull, distension of the sutures, reduced thickness of the skull bones and sometimes calcium deposits inside the tumor [4].

With respect to the pathological anatomy [5 8 19 23], the papilloma may show a considerable variation in size from the size of a pea to that of a mandarin orange. They are often soft, plastic and able to adapt their forms to that of the ventricles. Sometimes, though, they may erode and protrude into the cerebral parenchyma, which gives an impression of malignant infiltrating growth. But even if there is clinical malignity most choroid plexus papilloma show a benign histologi

cal picture, in principle in accordance with the ordinary structures of the choroid plexus. In a Swedish material of 11 cases [21], only one case is reported with definite criteria of histological malignity in the form of infiltrative growth. Hemorrhage edema and cysts are common in choroid plexus papilloma. Calcifications may sometimes occur to such an extent that the papilloma is visible in X ray pictures.

The choroid plexus tumors should be treated by surgical excision [1 4 25]. Treatment with X rays is of very little value for these in general cellular benign tumors. Cerebrospinal fluid-shunt operations may be of temporary value when the tumor obstructs the aqueduct [25]. The mortality in these operations reaches a total of about 50 % and for papilloma in the lateral ventricles about 25 %. Of Matson & Crofton s operated cases, 4 out of 15 died, 4 are reported to be retarded and 7 quit normal [18].

It is of importance for the prognosis that the choroid plexus papilloma should be diagnosed and extirpated in time. It has long been questioned whether hydrocephalus can be the result of over production of cerebrospinal fluid alone [22]. For this reason we consider ourselves justified in presenting and discussing an unusual case which began in the newborn period and which illustrates, firstly the difficulties of diagnosing choroid plexus papilloma in advanced hydrocephalus secondly that overproduction of cerebrospinal fluid alone seems to have been responsible for the hydrocephalus in this case and finally that the flow capacity of the Spitz Holter valve is insufficient when the production of cerebrospinal fluid is increased.

Case Report

The patient, a 3-month-old boy was admitted to hospital on account f rapidly progressing hydrocephalus. There was nothing of interest in his hereditary history His mother was healthy She had been exposed to German measles during the first three months f pregnancy but had probably had the disease as a child, so she was not taken ill. The birth, at the Luleå Maternity Clinic was normal, by vertex presentation at the calculated time Birth weight 3730 g. Skull circumference at birth 35 cm. H was lively at normally and developed normally for the first two months. Then his parents bserved the "sunset phenomenon as the first symptom and thereafter increasing skull growth. When 3 months old, he was found t be a clear case of expansive hydrocephalus t the Child-Welfare Clinic and was admitted to the Children Hospital in Boden. The circumference f the skull was then 49 cm. His psycho-motor development was retarded. He could fix his gaze but not grasp things. Neurologically there was clear spastic diplegia with clonus of the feet etc. Lumbar encephalography showed communicating hydrocephalus with very much enlarged lateral ventricles. The thickness of the brain parenchyma was measured as a little over 1 cm frontally as well as occipitally N enlargement of the third ventricle was observed. The passage through the aqueduct was free The cerebrospinal fluid was normal, no increase in cell content, and protein content was 55 mg %. Blood counts normal. Wasserman and toxoplasmosis titers negative.

When 4 months old, he was operated on t the Children Surgical Clinic in Uppsala with a ventriculo-caval shunt with normal-pressure valve *ad modum* Spit Holter and was given antibiotics. The skull growth continued nevertheless, and in spit f several revisions and a shunt operation on the ther side at the age of 6 months there was no sign f diminishing skull growth. The circumference was then 60 cm. At 7 months a new revision was performed at

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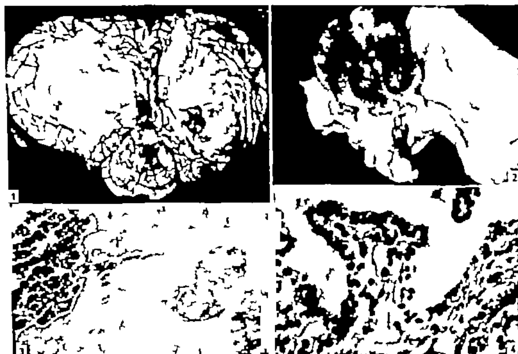


Fig. 1 Hydrocephalic brain with plexus papilloma protruding from the bottom of the wide right hand, lateral ventricle.

Fig. 2. The section surface of the tumor

Fig. 3. Microphotograph of the tumor tissue with extensive necrosis and vascular thrombosis. Weigert van Gieson, × 35.

Fig. 4 Higher magnification of papilloma structure Weigert van Gieson, × 540.

phalus progressed, however in spite of the fact that the shunt functioned well, as was verified at a later operation. Similar observations have been made by others [16] and motivated an investigation of the flow capacity of the Spitz Holter valve at different pressure gradients.

The Holter valve is manufactured in three different types [11] intended to hold a "restriction pressure" of 10 mm H_2O ("subdural" valve) ~5 mm ("medium") and 4 mm ("normal"). With a pressure gradient of 170 mm the "medium" valve gives 200 ml fluid in 4 hours but with a reduction of the pressure gradient to 70 mm it gives only 43 ml during the same period. The valve is thus very pressure-sensitive i.e. small variations in pressure can produce very large variations in flow. The pressure difference varies furthermore with the pressure on the outlet, in the superior caval vein and in the right atrium, where it is reported to vary between +100 and −60 during rest. For a more detailed discussion of these problems, see some recent papers by Forrest [10] and Macnab [16]. Neither the variations in volume of the normal cerebrospinal flow nor its upper limit under pathological circumstances are known at present. The data cited above concerning the flow capacity of the valve

give reason to supect that it may in some cases be below the cerebrospinal fluid production and that the combination of increasing hydrocephalus and functioning valve may thus be explained.

In secondary infection with persisting bacteremia in a functioning Holter valve one may be forced to exteriorize the caval catheter These cases might provide a clue to the volume of cerebrospinal fluid production and its variations. The case discussed above can be considered to show that choroid plexus papilloma contribute to an abnormally raised cerebrospinal fluid production and that the Holter valve does not then always give adequate drainage

Summary

Choroid plexus papilloma occur with a frequency of less than 4 % of all intracranial tumors in childhood. In children they usually give hydrocephalus and xanthochromous cerebrospinal fluid with a rise in protein content A case is presented illustrating the difficulties of diagnosing choroid plexus papilloma in advanced hydrocephalus. Over production of cerebrospinal fluid seems to have been responsible for the hydrocephalus in this case. Insufficient flow capacity of the Holter valve when the production of cerebrospinal fluid is increased, is discussed.

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PROGRESS IN PEDIATRICS

Congenital Neutropenia A Report of Five Cases

by J B MacGILLIVRAY J V DACIE, J R. K. HENRY L. S. SACKER and J P M TIZARD

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In 1956 Kostmann [13] described in a remarkable publication what he considered to be a new disease which he named *Infantile Genetic Agranulocytosis* Fourteen cases were observed in an isolated community in North Sweden and detailed studies were made of six cases in which adequate material was available In the other eight cases the diagnosis was assumed from the children s history

The children Kostmann observed were the off-spring of nine families, in five of which the parents were related. Of the six cases studied, four died at an early age; two were alive at the time of reporting aged 5 and 4½ years of age respectively The presenting feature was skin sepsis at 1 week, 3 weeks, 1½ *½ 3 and 5 months. There was persistent severe neutropenia and the bone-marrow showed arrest of neutrophil granulopoiesis at the promyelocyte and myelocyte stage Other features described in some of the cases were an increased number of eosinophils in the bone marrow an eosinophilia and monocytosis in the peripheral blood a high platelet count and a raised serum gamma globulin. As a result of family studies Kostmann concluded that the condition was inherited as an autosomal recessive the high incidence in the population he studied being due to intermarriage

Since Kostmann s original description, a number of cases showing similar features have been reported (see discussion)

In recent years five children suffering from neutrophil agranulocytosis or severe persistent neutropenia of unknown aetiology and showing many of the features described by Kostmann have been seen in this hospital. Three died in infancy two are alive in early adolescence but are still neutropenic. One of our cases, Case –, was reported briefly by Henry [9].

Case 1 A.S

Female child born 8th August, 1954 died 29th August 1956.

She was the second child of healthy unrelated parents. The first child is alive and well. Pregnancy and delivery were normal.

She was first admitted to the Hammersmith Hospital on 30th November 1955.

Past History At the age of 8 days she developed a septi right thumb and mild umbilical sepsis which took 5 weeks to heal in spit of treatment with chlortetracycline and penicillin.

Subsequently she was admitted to another hospital at the ages of 7 weeks, 11 weeks,

4 months, 5 months, 11 months and 15 months. All the admissions were for pyogenic infections which included otitis media, multiple septic spots, tonsillitis and an infected vaccination sit with cellulitis. She was treated for these at varying times with penicillin, chlortetracycline erythromycin and streptomycin. The first full blood count when she was 5 months old, showed.— haemoglobin, 10.1 g/100 ml, leucocytes, 8100/mm with only 4% neutrophils (3*4/mm³). Serum proteins, electrophoretic pattern, and X-rays f long bones were normal. Examination f the bone-marrow showed average cellularity with a striking reduction of cells f the neutrophil granulocyt series and considerable increase in monocytes. Subacut monocytic leukaemia was diagnosed.

Her condition deteriorated with loss f weight persistent fever anaemia and hepatomegaly She was transfused twice and on the 6th April 1955 she was started on cortisone The initial ffect f this was striking; her temperature subsided and she gained weight. When discharged 3 weeks later she was af brile and much improved. A blood count at this time showed.— haemoglobin, 11 7 g/100 ml; leucocytes, 14,800/mm 1 eosinophils (1776/mm³), 26 monocytes (3830 mm³), 62 lymphocytes (9200 mm).

There were two more admissions (at 11 months and 15 months) for tonsillitis and otitis media. Examination f her blood during these admissions howed the ppearances t be substantially unchanged. X-rays of the skeleton t the age of 11 months showed no bony bnormality She was continued on cortisone 3 mg dail apart from a 10 day period when ACTH 3 units daily was substituted.

She was admitted t the Hammersmith Hospital, aged 16 months, for a further opinion. She wa found t be poorly nourished and to weigh 7.9 kg. There was an offensi serous discharge from the right ear her mouth was dry and there w marked hypertrophy f the gums. The fauce were inflamed and there were small firm lymph nodes palpable in both posterior cervical triangles, both axillae and inguinal regions.

The bdomen was prot berant the li er was enlarged 9 cm below the costal margin in the midclavicular line· the spleen was just palpable Other systems were clinically normal. A blood count showed a moderately severe anaemia: haemoglobin 4 g/100 ml, total leucocytes, 20 000/mm³ 9 % eosinophils (1800/mm³) 1 basophils (200/mm³), 57 % lymphocytes (11,400/mm³) 33 monocytes (6600/mm³)

Before further investigations the cortisone was stopped. She was transfused with 200 ml f packed cells and following this her haemoglobin rose to 11 9 g/100 ml. During the entire 5 weeks admission he ran swinging temperature which varied between 36.1 and 40°C. This was not affected by tetracycline given to cover a bone-marrow puncture which wa carried out in the operating theatre This showed normal erythropoiesis but very depressed neutrophil granulopoiesis. Eosinophils were relatively conspicuous (T ble 1) There was no ill effect from this bone-marrow puncture and she was discharged.

Both parents had normal blood counts and her mother showed a normal rise in her neutrophil count fter injection f T.A.B. vaccine

A diagnosis f congenital agranulocytosis was made and it was felt wisest to restrict ttempts t therapy t the use of antibiotics required, and blood transfusion.

She w seen in the out-patient department in February and March 1956, and on both occasions her condition was fairly satisfactory On the latter isit she was found t have gained weight (8.9 kg)

She was readmitted int Hammersmith Hospital in May 1956 aged 1 months. She had remained f irly well until 2 weeks previously when he had caught cold. Since then she had been irrit ble anorexic and had run continuous temperatur

On examination she had watery discharge from her right ear and nose and moderat enlargement f cervical, axillary and in

Table 1 *Bone-Marrow Findings in Five Cases of Congenital Neutropenia*

Case	1 A.S. %	2 S.B. %	3 R.H %	4 G.P %	5 S.H.
Reticulum cells			5		0.8
Myeloblast	4	0	3	4	2.4
Promyelocytes	2	3		1.9	4 6
Myelocytes					
neutrophil	1			9	9.8
eosinophil	4	4	2	9	7.6
Metamyelocytes					
neutrophil				0.3	2.8
eosinophil	3	8		2.0	
Polymorphonuclears					
neutrophil	0.2			1	
eosinophil	13	18		4	10.8
Lymphocytes	30	48	65	41	19.8
Monocytes	13		10	2	
Plasma cells	1 7	1		1	4 6
Normoblasts	28	15	15	4	37

guinal lymph nodes. The abdomen was protuberant and tympanitic and the liver and spleen were enlarged as on the previous admission. Her chest was clinically clear. The heart was not enlarged but there was a short grade-II systolic murmur loudest at the base.

Culture of the discharge from the ear yielded *P. pyocyanea* the organism was sensitive to sulphathiazole and partly sensitive to tetracycline and she was treated with a combination of penicillin and tetracyclin. Six days after admission signs of consolidation developed in the upper zone of the right lung and a chest X-ray showed consolidation of the right upper lobe and apical segment of the lower lobe. A laryngeal swab grew *Staph. pyogenes* sensitive to erythromycin only.

Her serum proteins totalled 8.8 g/100 ml albumin 3 0 g/100 ml globulin 5 8 g/100 ml. Electrophoresis showed a marked increase in the gamma globulin (Fig. 1). She was transfused with 200 ml of fresh blood. Few if any of the donated neutrophils circulated, only 1 in 200 of the leucocytes was a neutrophil 2 hours after the transfusion and none was seen in 500 cells 18 hours post transfusion.

Three weeks later the signs in the right lung were still present and she had developed marked clubbing of her fingers and toes. She was extremely wasted and anorexic and refused to take any solid food, but in spite of this her caloric intake was maintained at about 1,500 calories per day by using milk fortified with glucose. Complan

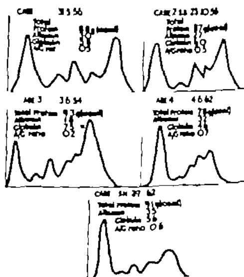


Fig. 1 Filter paper electrophoresis of serum proteins.



Fig. 2. Case 1 A.S. X-ray of chest showing extensive consolidation of the right lung.

and Casilan. She was also given 10 mg f Androstalone daily in an ffort t improve her nutrition. This had no side-effects, but on the other hand there was no gain in weight.

The chest condition became progressively worse and the chest X ray showed the right upper and lower lobes to be consolidated (Fig.) Her bdomen was distended and the liver remained enlarged. Cultures from her skin, nose etc. repeatedly grew *Staph pyogenes* Combinations of antibiotics were used but there was no clinical improvement. She finally died on 29th August 1958, aged 2 years.

Post Mortem Exam nation

She was a pale emaciated child with heavy and thickened limb bones.

There was consolidation f the right upper lobe with numerous abscesses up to 3 cm in diameter containing thick yellow material. The pical segment of the right lower lobe was similarly affected (Fig. 3) Sections showed interstitial fibrosis and areas of necrosis with a surrounding cellular infiltrat consisting mainly f numerous plasma cells.

The liver w ighed 944 g and the sinusoid were congested. In the kidneys there were

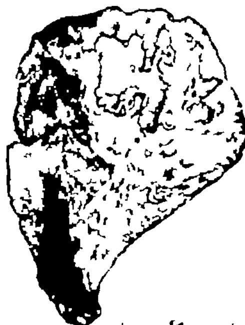


Fig. 3. Case 1 A.S Right lung showing large necrotic lesions.

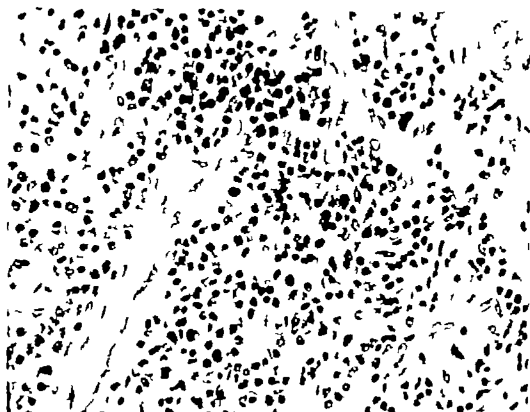


Fig. 4 C se 1 A.S. Section of gum showing cellula infiltration in which plasma cells are conspicuous (H and E. 500)

small wedge-shaped subcapsular collections of l mphocytes, plasma cells and occasional eosinoph ls. Th spleen weighed 40 g The Malpighian bodies were of normal size The pulp w fibrotic and contained large amount of haemosid rin and many plasma cells. The liver spleen and kidney showed a negative reaction on testing for myloid There was moder t generalized lymph node enlargement Th nodes cont ined large amounts f haemosiderin and many plasma cells in the medullae The femoral, tibial and vertebral marrow was cell lar and cont ined numerous eosinophils, but n neutroph l were identified. The gums were hypertrophied and heavily inf ltrated with plasma cells (Fig. 4)

The right femur and tibia were removed. Both were greatly thickened due t the formation of a thick layer of subperiosteal bone the appearance bei g that of gross secondary (pulmonary) hypertrophic osteoarthropathy (Fig 5 and 6)

Case 2 S.B

Female infant Born 1st M y 1956, died 1 th January 1957

Mother unmarried. Normal full-term delivery; birth weight 3 kg

At the age of 14 days the infant developed a serous discharge from the left ear profuse nasal discharge and bilateral conjunctivitis. She was admitted to Hammersmith Hospital on 10th May 1956.

On examination she was seen t be sick infant her temperature wa 38.3 C. There were few abnormal physical signs. However the posterior cervical lymph nodes were en-

larged, the umbilicus was moist and there was profuse watery discharge from the nose and left ear

Investigations

Haemoglobin, 16.3 g/100 ml; total leucocyt count 8000/mm neutrophils 1% (80/mm³) eosinophils 27% (2160/mm³), lymphocytes 41% (3280/mm³), monocytes 3 „ (2480/mm³).

Cultures taken from the throat left ear and nose grew *Staph. pyogenes* sensitive t penicillin.

A chest X ray was normal.

Puncture f the t bia showed an almost complet bsence f neutrophil granulopoiesis, a small increase in blast and an increased number f eosinophils and eosinophil precursors (Table 1)

A diagnosis f congenital agranulocytosis w made and symptomatic treatment commenced with intramuscular penicillin, 250 000 units 8-hourly The discharging left ea slowly dried up. However after bout 3 weeks septic spots ppeared over the left preauricular region, followed by enlargement f the left submandibular gland. At this time umbilical sepsis also developed. Cultures grew *E coli.*, *Strept faecalis* and a *Prote*



Fig. 5. Case 1, A S Femur tibia and fibula showing ery marked subperiosteal new bone formation.



Fig. 6. Case I A.S. Section of the t bia showing very marked subperiosteal new bone formation (H. and E., 6.2).

TABLE 2 *Haematological Data in Five Cases of Congenital Neutropenia*

Case	Haemoglobin Minimum, g/100 ml	Reticulocytes Maximum, %	Total leucocytes Range, mm³	Neutrophils range %	Neutrophils range mm³	Eosinophils range %	Eosinophils range mm³	Basophil %	Lymphocytes range %	Lymphocytes range mm³	Monocytes range %	Monocytes range mm³	Platel. Ran. mm³
A.S.	7 1	3	8,000– [illegible]0 000	0– 3	0– 4[illegible]0	4–34	850– 5,780	0–3	38–84	2,800– 14,500	7–44	560– 6,600	[illegible]
S.B.	7 7		3,000– 8,000	0–	0– 100	8–44	390– 3,520	0–3	41 88	–200– 7,000	1 31	60– 2,450	70,[illegible]
R.H.	7 4		3,000– 12,000	0– 4	0– 2,100	0–14	0– 1,056		54–92	2,700– 10,200	2 4	60– 1,920	–
G.P	8.6	–	–700– 8,600	1 24	64– 1,664	1 12	33– 936	0–1	34–97	1,280– 7,000	2–42	200– 2,400	[illegible]
S.H.	9 6	0.8	–400– 3,100	0–1	0– 284	0– 6	0– 144		64–98	1 600– –070	11–33	40– 890	[illegible]

The *Strept faecal* was sensitive to chloramphenicol and erythromycin and the *Proteus* to streptomycin, chloramphenicol and sulphathiazole

She then developed cellulitis of the right side of the front of the neck around the sit of vein puncture The general condition worsened, she appeared toxic and developed a high temperature The swelling in the neck was in cised and some thick greenish yellow material released. *Staph pyogenes* sensitive to penicillin was grown. On d rect examination only a few monocytes and no pus cells were found Following incision of the swelling her condi tion improved and the septic lesions cleared up. She was discharged on the 14th August, 1956 weighing 5.4 kg Apart from penicillin she had received the following antibiotics and chemotherapeutic agents: chlortetracycline sulphafurazole streptomycin, tetracycline and erythromycin. Chloramphenicol int ment and papain, .5% in starch were pplied to the umbilicus.

The child continued to attend hospital because of continuous and resistant umbilical sepsis. At this time her urinary amino acids were found to be of a normal pattern; her serum proteins totalled 6.. g/100 ml, albumin 2.3 g globulin 3.9 g. Electrophoresis showed a marked increase in the gamma globulin (Fig. 1)

She was readmitted on the "6th September 1956 with acute tonsillitis and umbilical sepsis. No organisms were grown from a throat swab, but a culture from the umbilicus grew *Strept faecalis* sensitive only t chlor amphenicol. She was therefore treated with chloramphenicol, 30 mg 4-hourly She responded well to this, her temperature subsided and she was discharged on the 4th October 1956. However the umbilicus was still severely infected and she continued to be seen as an out patient An ulcer on the left side of the cheek was treated with tetracycline

Acute tonsillitis and an abscess of the right buttock necessitated two further admissions before the final admission on the 21st December 1956. She then had an abscess on the right side of the neck and more chronic infection of the left cheek. X-ray f long bones showed no periosteal reaction. She was given courses f streptomycin, erythromycin, chloramphenicol, novobiocin and sigmamycin. Her condition, however deteriorated and massive cellulitis of the left groin developed which extended down t the left labia and perineum; she

ran a high fever and died on the 17th January 1957

At no time during the course of her illness had there been more than 100 neutrophils per mm On many occasions no neutrophils were seen. The eosinophil count was continuously raised, the highest recorded count being 3520/mm The monocyt count fluctuated widely (Table 2).

Post Mortem Examination

She was a pale child with inflamed areas over the left cheek, right side f the neck, left iliac crest and perineum.

There were widespread haemorrhagic areas in both lungs, with the shape and con sistency f recent infarcts. Sections showed focal areas of intense intra-alveolar haemorrhage containing clump of gram-positive cocci with very little surrounding inflamma tory reaction. There was slight plasma-cell infiltration round the bronchioles.

Both kidneys were pale. There was peri glomerular plasma-cell infiltration and a more diffuse infiltration of the interstitial tissue with plasma cells. The spleen weighed 50 g: the Malpighian bodies could be seen macroscopically Section showed numerous plasma cells in the pulp.

There was moderate generalized lymph adenopathy and sections of one of the nodes showed large amounts of haemosiderin and many plasma cells in the medulla.

The bone-marrow was cellular with many eosinophils and numbers of plasma cells. N neutrophils were seen. Section of the skin from the perineum showed ulceration and necrosis, with virtually no inflammatory reaction and many gram-negati bacill on the surface

Case 3 R.H

Male infant, born August 1953 died 25th August 1954. Birth weight 3.5 kg; the infant was delivered by Caesarean section for disproportion.

The infant father was healthy; the mother suffered from petit mal, but did not take any anticonvulsants or sedatives during her pregnancy She had had one pre vious miscarriage and one ectopic gestation. One sibling died at theage of 3 months f "bronchopneumonia" Repeated blood counts on both parents were normal.

The infant had suffered from continuous episodes f sepsis since the seventh day f life. These took the form of a discharge from the umbilicus in the neonatal period, boils and small subcutaneous abscesses on the scalp and body and paronychia. At the age f 7 months he developed bilateral otitis media. This was complicated 1 month later by right-sided mastoiditis which required operation. *Staph pyogenes* was isolated from all these septic lesions and over the course of time the organism became increasingly resistant to the various antibiotics used in treatment.

The first blood count was performed at the age f 7 weeks. The total leucocyt count was normal, but only % of the cells were neutrophil polymorphs. Numerous subsequent blood counts showed the same pat tern, usually no neutrophils were present but there was one count of 4% (100/mm³). A marrow puncture at the age of 8 months was reported as showing a marked depression of neutrophil granulopoiesis but other wise no abnormality and the diagnosis re mained in doubt.

The infant was admitted t Hammersmith Hospital in June 1954 at the age of 10 months. On admission he did not ppear gravely ill. His weight was 5.8 kg. Numerous (pproximately 50) furuncles and subcuta neous " bscesses were present over the back, buttocks, both thighs and scalp. There was a discharging sinus over the right ma stoid scar The regional lymph glands were enlarged. The spleen was palpable cm below the costal margin.

I vestigations

Haemoglobin, 9.5 g/100 ml total leucocyt count 8000/mm³; eosinophils 6 (480/mm) lymphocytes 70% (5600/mm³); monocytes 4 (1920/mm³)

Serum proteins: total 9.3 g/100 ml; albumin 1.8 g; globulin 7.5 g Electrophoresis

TABLE ⁰ *Haematological Data in Five Cases of Congenital Neutropenia*

Case	Haemoglobin Minimum, g/100 ml	Reticulocytes Minimum, %	Total leucocytes Range mm³	Neutrophils range %	Neutrophils range mm³	Eosinophils range %	Eosinophils range mm³	Basophils %	Lymphocytes range %	Lymphocytes range mm³	Monocytes range %	Monocytes range mm³	Plat/ Rng mm³
1 A.B.	7 1	3	5,000– 20 000	0– 3	0– 420	4–34	540– 6,780	0–3	38–84	2,660– 14,200	7–41	540– 6,600	[illegible]
2 S.B	7 7		3,000– 8,000	0– 2	0– 100	8–44	380– 3,520	0–3	41–88	2,200– 7,000	1–31	50– 2,480	[illegible]
3 R.H.	7 4		3,000– 12,000	0– 4	0– 2,100	0–14	0– 1,056		64–92	2,700– 10,200	2 24	60– 1,830	–
4 G.P	8.6		2,700– 8,600	1 34	64– 1,564	1 13	33– 936	0–1	34–97	1,380– 7,000	2–41	200– 2,459	[illegible]
5 S.H.	9.6	0.8	2 400– 3,100	0–12	0– 384	0– 6	0– 144		64–96	1 600– 2,070	11–33	240– 880	[illegible]

The *Strept faecalis* was sensitive to chloramphenicol and erythromycin and the *Proteus* to streptomycin, chloramphenicol and sulphathiazole

She then developed cellulitis of the right side f the front of the neck around the site f vein puncture The general condition worsened, she ppeared toxic and developed a high temperature The swelling in the neck was incised and some thick greenish yellow material released *Staph. pyogenes* sensitive to penicillin was grown. On direct examination only a few monocytes and no pus cells were found. Following incision of the swelling her condition improved and the septic lesions cleared up. She was discharged on the 14th August, 1956 weighing 5.4 kg Apart from penicillin she had received the f llowing antibiotics and chemotherapeutic agents, chlortetracycline, sulphafurazole streptomycin, tetracycline and erythromycin. Chloramphenicol ointment and papain 25% in starch, were applied to the umbilicus.

The child continued t attend hospital because of continuous and resistant umbilical sepsis. At this time her urinary amin acids were found to be of a normal pattern; her serum proteins totalled 6. g/100 ml, albumin 2.3 g globulin 3 9 g. Electrophoresis showed a marked increase in the gamma globulin (Fig. 1)

She was readmitted on the 26th September 1956 with acut tonsillitis and umbilical sepsis. No organisms were grown from a throat swab, but a culture from the umbilicus grew *Strept faecalis* sensitive only to chloramphenicol. She was therefore treated with chloramphenicol 30 mg 4 hourly She responded well to this, her temperature subsided and she was discharged on the 4th October 1956. However the umbilicus was still severely infected and she continued to be seen as an out patient. An ulcer on the left side f the cheek was treated with tetracycline.

Acute tonsillitis and an abscess of the right buttock necessitated two further admissions before the final admission on the 21st December 1956. She then had an abscess on the right side of the neck and a more chronic infection of the left cheek. X ray of long bones showed no periosteal reaction. She was given courses of streptomycin, erythromycin, chloramphenicol, novobiocin and sigmamycin. Her condition, however deteriorated and massive cellulitis of the left groin developed which extended down to the left labia and perineum; she

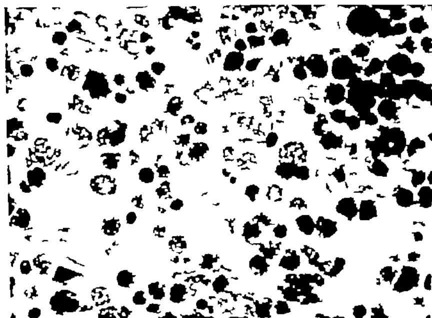


Fig. 7 Case 3, R.H. Post mortem section of bone marrow showing numerous eosinoph ... (× 750)

clear but she was still pyrexial. PAS 0.5 g t.d.s. was given and this was increased to 1 g t.d.s. after 1 month but was stopped shortly after. During this period she developed a septic blister on the foot which was treated with penicillin. She then developed an attack of acute tonsillitis which was treated with sulphamezathine. A leucocyte count after commencing this drug showed 5400 cells/mm³ with 9% neutrophils (480/mm³). In view of the neutropenia the sulphamezathine was stopped after 7½ g had been given. The sternal marrow was reported as showing arrest of neutrophil granulopoiesis. Folic acid and pyridoxine were given without effect.

Tonsillectomy was carried out on the 3rd August 1950 under penicillin cover. One pint of blood was given post-operatively and she was discharged in a satisfactory condition. Blood count on discharge showed the neutropenia to be persisting (Table).

She was seen on the 1st April, 1951 by one of us (J.V.D.). The presence of neutropenia was confirmed and bone-marrow puncture was carried out (Table 1). T... showed depression of neutrophil pre... cess accompanied by active ... tion. There appeared to be ... leukaemia and diagnosis of ... penia of unknown origin, ... made.

Blood count carried ... parent and on her brother ... lues, and no abnorma... in films.

Further Progress

This is summarized b... 1951 1954 several p... cert in aetiology 19... X-ray showed bilater... abscess of right ... Odstock Hospital at ... d pyrexia and cough ... ry opacity in the right ... ase with penicillin and ... electrophoresis sho... um and ... reed pathology ... logy

On the 2nd Dec... er of

mitted to the Hospital for Sick Children, Great Ormond Street, under the care of Dr A. P Norman. Physical examination then showed a thin girl of 11 yrs. 3 months with bilateral cataracts. Her weight was 23.. kg; her height 1"8.9 cm. There were no other physical signs of note. X ray of chest showed infiltration and possible cavitation of the right upper lobe and the apical region of the lower lobe, probably secondary to staphylococcal pneumonia.

The blood picture and bone-marrow appearances were as before (Tables I and). The serum proteins totalled 7 7 g/100 ml on electrophoresis the α and α_2 globulins were increased and the gamma globulin was markedly increased. She was discharged on the "0th December 1958.

She has had no illnesses since this time apart from an occasional cold and had a normal menarche at the age of 13. She has seldom been absent from school and is making good progress with her work. Her father states that small pustules on her face which formerly produced a wide area f reddening and no "head now show a pocket of pus. However the most recent blood count (4th June 1962) showed that she was still markedly neutropenic (Table). Electrophoresis revealed a raised serum globulin content, particularly f β and γ globulins (Fig. 1).

Case 5 S.H

A female born 21st October 1951 She is the second child of non-consanguineous parents. Pregnancy was uneventful and delivery normal and full term. The birth weight was *5.1* kg.

As an infant she was quite well until about 3½ months old when she began to have crops of boils on her perineum She was then admitted to Oldchurch Hospital under the care of Dr T Savage for investigation and was found to have a marked neutropenia. She remained well until 8 months, when she developed blister like lesions on her tongue, the inside f her cheeks and on her lower lip. These were filled with clear non purulent fluid and have subsequently reappeared at 4–6 weekly intervals ever since. She also had minor infections of the eyelids, tonsils and skin. Steroids and most of the known haematinics were tried without effect. In 1955 she was found to have a serum protein level of 9 g/100 ml; albumin, 4 1 g, globulin 4.9 g, with a raised content of gamma globulin. A bone-marrow examination in 1960 was reported as showing very scanty mature granulocytes. She remained well, however in spite of numerous septic episodes and made an uneventful recovery from chickenpox, whooping cough and measles. Routine vaccinations and inoculations were performed without complications.

She was admitted to Hammersmith Hospital on the .8th January 1962.

Physical Examination She was a pleasant co-operative child, below the 3rd percentile for height and just on the 3rd percentile for weight. Her teeth were carious and discoloured, the gums hypertrophic and receding with extensive gingivitis. Her tonsils were huge and the crypts contained white material, the anterior cervical lymph nodes were slightly enlarged. There were scars of skin lesions on the neck, back and thighs but no other physical findings of note.

X rays of chest, skull and long bones were normal.

Laboratory Findings

The results of blood counts are summarized in Table .., the most striking feature being the severe and sometimes total neutrophil granulocytopenia. At no time has her absolute neutrophil count been higher than 384/mm The highest percentage of monocytes counted had been 22% (890/mm³) and eosinophils 6% (144/mm³) She has always had a mild normochromic anaemia and a haemoglobin of about 10 g/100 ml. Her platelet count on several occasions has been in the region f 700 000 / mm

Her father mother and 15-year-old sister had normal blood counts.

The serum proteins totalled 9 1 g/100 ml; albumin 3 5 g, globulin 5.6 g, and electrophoresis showed a raised gamma globulin.

Tests were kindly carried out by Dr K. L.

G Goldsmith of the Lister Institute for leucocyt or platelet antibodies but none were demonstrated. Dr E. A. Herbert f the Bernhard Baron Institut of Pathology The London Hospital, examined her chromosomes, which showed a normal pattern.

There was a slight general increase in urinary amino acids.

Bone-marrow examination showed a hypercellular marrow with normal erythropoiesis, but with relative depression f neutrophil granulopoiesis. There was on the other hand an increased number of eosinophils and eosinophil precursors (T ble *).

Further Progress

She was referred to the Dental Department (Miss Whitehead) for treatment of the gingivitis. While in hospital she developed an ulcer on the lower lip similar t those she had in the past No pus formation was noted and the ulcer healed slowly She was discharged on the 22nd February 1962.

Discussion

'Idiopathic" neutropenia is a rare condition at any age It is certainly rare in infancy and childhood although a number of varieties have been described.

Transitory granulocytopenia in the new born was described by Slobody Abramson & Loizeaux [*1]. Stefanini, Mele & Skinner [23] reported two similar cases; the mothers of the children were neutropenic and in one case leuco-agglutinins were demonstrated in the blood of the mother In the cases reported by Brown *et al* [4] maternal leucocyte antibodies were also found.

Cyclic neutropenia has also been noted in the first year of life [17 18 19].

Stahlie [22] who described a case of *chronic benign neutropenia* occurring in an 8-month-old female child collected 16 similar cases from the literature Thirteen of the children later developed normal counts. One however died of tuberculosis and in two cases the granulocytopenia persisted. The children had a lowered resistance to infection and bone marrow examination when performed showed an increased number of stab cells and myelocytes often not apparent until a differential count was done

A benign form of *familial neutropenia* has been described among members of eleven families of Yemenite Jews [6]. Bone marrow examination, when done did not show any evidence of maturation arrest and the condition was asymptomatic.

Kostmann s cases clearly differ from the above in their chronicity the high mortality and in the marrow appearance of depression of granulopoiesis.

Since his original description a number of cases showing similar features have been reported, some occurring in siblings Lethal congenital neutropenia with eosinophilia was described by Andrews, McClellan & Scott [2] in two siblings who died at the ages of 4 and 5 months; they suggested a recessive inheritance Bjure Nilsson & Plum [3] reported two boys aged 2 and 7 in a family of four who were neutropenic and prone to infections. Two siblings with neutropenia were reported by Rosman & Hummer [20], one died aged 9 the other was alive aged 20 at the time of reporting; their cases lack some of the features described by Kostmann, and A.C.T.H injection produced a temporary rise in neutrophils in their surviving case

Single fatal cases similar to Kostmann with no evidence of hereditary aetiology have been described by number of

authors [1 8 12 14] McLean [15] reported a case in 1937 this child is alive and well (196–) although still neutropenic [10]. In none of the cases was there any evidence of consanguinity

Finally a family was described by Hitzig [10] in which the neutropenia appeared to be inherited as a dominant The father aged 30 son aged 8 and daughter aged 4 were all neutropenic. The blood and marrow findings were similar to those in Kostmann's cases, although clinically severe infections were not a feature

The five cases described in this paper have many features in common both from the clinical and haematological points of view It seems reasonable to attribute the children s inability to combat the coccal infections to the severe and sometimes total neutropenia and it is interesting to contrast with this the fact that one at least, of the children (S H) managed to make satisfactory recoveries from whooping cough chickenpox and measles

The raised levels of serum gamma globulin, which all the children showed, are presumably a response to the long continued infection

It is likely that our patients have suffered from congenital severe and persistent neutropenia. Although the earliest blood counts were not done until 2 weeks of age it is reasonable to assume that this was present since birth In Case 4 the first count showed 1504 neutrophils/mm³ and on two subsequent occasions the total neutrophil count was apparently within normal limits. It is possible that the neutropenia in this child was drug induced On this hypothesis it is remarkable that the neutropenia has persisted for 13 years subsequently In other respects, although less severely affected, she showed similar features to the others.

Our cases provide no evidence as to a possible hereditary aetiology but as already mentioned, they strongly resemble both clinically and haematologically the cases described by Kostmann in which inheritance as an autosomal recessive seemed probable

As will be emphasized below four out of the five patients had persistently raised eosinophil counts and the fifth patient a normal eosinophil count despite a marked neutropenia or absence of circulating neutrophils Kostmann s title "infantile genetic agranulocytosis" is thus a misnomer and "infantile genetic neutrophil agranulocytosis (or neutropenia)" is preferable.

The blood and bone marrow pictures of all our patients have been similar although varying in degree and they have been markedly similar to those of Kostmann s cases. The typical blood film showing marked rouleaux formation, paucity or absence of neutrophils excess of eosinophils and monocytes and possibly of platelets too provides an unmistakable picture Few if any abnormal cells (myelocytes promyelocytes or blasts) circulate and the blood picture should not be confused with that of leukaemia Anaemia is frequent and may be moderate in degree It seems likely that this can be attributed to the effects of chronic infection

The post mortem findings in the three fatal cases illustrated the consequences of chronic skin and pulmonary sepsis. Sections showed a complete lack of neutrophils in the lesions and an excess of plasma cells The extreme degree of secondary (pulmonary) hypertrophic osteoarthro-

pathy in Case 1 was a notable feature (Figs 5 and 6)

In Case 5 superficial scarification of the skin over the flexor aspect of the forearm was carried out so that the tips of the dermal papillae were just exposed. In 3 hours, only scanty mononuclear cells were found in the exudate compared with numerous neutrophils found in a normal control after the same time

In the fatal cases of our series recurrent surface infections (boils and otitis media) dominated the clinical picture from early infancy A striking feature was that the discharge from boils and middle ear infections was clear and watery rather than purulent. Later some of the children developed internal infections, particularly chronic pneumonia and it is interesting to compare the sites of primary infection with those commonly found in agammaglobulinaemia in which although skin sepsis may be present internal infections such recurrent pneumonia, arthritis and meningitis re often early manifestations [3 11].

The exact mechanism of this type of congenital neutropenia has not been established. As it appears to be inherited as a simple utosomal recessive character presumably there is a single enzyme defect

The rise in eosinophil and monocyte counts, which is so characteristic of most f these patients, is an interesting phenomenon Possibly the cells can play some part in combating infection, although presumably less ffectively than do neutrophils, and the eosinophilia and monocytosis may thus be response to the infection. Alternativel it is a possible hypothesis that th cells are formed in excess imply beca se their precursors being unable to carry out the normal transformation to neutrophils differentiate along alternative lines. An analogy might be drawn with another autosomal recessive disease—the adrenogenital syndrome—in which the failure of synthesis of hydrocortisone leads to excess production of ACTH with consequent stimulation of the adrenal to produce androgenic hormones. It is possible that the negative feed back control of a hormone concerned in the production of both neutrophils and eosinophils is provided by a metabolic product of neutrophils alone It would be of interest to measure the eosinophil count of patients with congenital neutropenia transfused with neutrophil polymorph The high platelet counts seen in all of our patients where counts are available may gain be the automatic consequence of a block of the path along which the bulk of the myeloid cellular ctivity normally develops

In one of the two cases reported by Bjure Nilsson & Plum [3] transfusion of fresh plasma caused a t mporary rise in the number of neutrophils from (336 mm) to 4 (3400 mm) in eight days. In both their cases the addition of fresh plasma to cell cultures of the bone marrow produced an increase in granulopoetic activity The addition of cysteine had no effect

They suggest that deficiency of a plasma factor wa responsible for the neutropenia

In contrast Plum (quoted by Kostmann [13]) who studied cultures of the bone marrow f one of Kostmann patients reported that igns of granulocyt matu ration could be detected when the patient marrow cells were grown in normal serum and that maturation was more

marked when cysteine was added. Similar findings were reported by Hedenberg [8] who like Kostmann [13], concluded that the disease was due to a congenital inability to utilize S-containing amino-acids. Treatment with cysteine however was ineffective in both Kostmann's and Hedenberg's patients.

The blood-cysteine level was estimated in Cases 1 and 2 of our series The levels were 3 mg and 2.5 mg/100 ml respectively A normal control gave a level of 1.85 mg/100 ml. The significance of these figures is uncertain and might only indicate poor liver function Case 2 was treated with cysteine 0.5 g t d.s by mouth for 2 weeks with no effect on the neutrophil count

All the known haematinics have been tried in this condition without any observable benefit Treatment at present therefore consists in combating infections as they arise with suitable antibiotics. It seems probable that in the past before the introduction of antibiotics children with this disease died before a diagnosis was made

Summary

Five cases of severe neutropenia of probable congenital origin are described. Severe skin sepsis was a prominent clinical feature The peripheral blood often showed a monocytosis, eosinophilia and sometimes a raised platelet count The bone-marrow findings were depression of neutrophil granulopoiesis and prominence of eosinophils All the cases had a raised serum gamma globulin. Three died in early infancy two are alive in early adolescence but are still neutropenic

The clinical and haematological features were similar to "infantile genetic agranulocytosis" as described by Kostmann.

The condition varies in severity and some patients given suitable antibiotic therapy may survive into adolescence

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R. Haubrich Klinisch Röntgendiagnostik innerer Krankheiten I Thorax.

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This volume which is part of a series of monographs in medical radiology deal with the normal and pathologic roentgen findings of the heart and great vessels, the lungs, pleura mediastinum, and the diaphragm. It gives an extensive but concise review of modern knowledge and also includes discussions of the current dynamic and rapidly expanding aspects of diagnostic radiology. The different method of investigation are excellently described and the chapters on *normal roentgen anatomy deserve special* praise for their clarity. It is noteworthy that the roentgen findings are correlated with appropriate clinical data, and that the results of some laboratory investigations, particularly *cardiac catheterization*, are given due reference in the text. The volume is richly illustrated and the illustrations are as a rule of a high quality some of them being superb. From the viewpoint of the pediatrician or the pediatric roentgenologist more adequate coverage for diseases of children would be desirable but perhaps its omission is unavoidable in a text of this type. However the chapter on tuberculosis of the lungs is the most rewarding. Ample illustrations support the clear and informative text. The pathology of the child lung is otherwise only sparsely dealt with. The section on congenital heart lesions, although splendid in some respects, is not of the same general high standard as many of the other chapters, the roentgen description used being unnecessarily defined by terms which are influenced by old clinical concepts. Also with some excellent exceptions the angiocardiograms *have not been reproduced well. In several of* those it would have been advantageous to supplement the angiocardiograms with explanatory drawings.

The over all standard of this volume is, however high and the limitations mentioned should not detract from the fact that it represents a valuable contribution to modern to have been selected with great care. The book can be warmly *recommended even to* the experienced roentgenologist.

Ulf Rudhe

Minimal Cerebral Dysfunction. Ed. by M. Bax & R. McKeith.

Published by the National spastics society medical education and information unit in association with W. Heinemann Medical Books Ltd London 1963. Price 17.6s.

The concept of minimal brain damage or minimal cerebral dysfunction has caused considerable confusion with many different interpretations. Under this heading varying categories of neuropsychological abnormalities have been listed such as cases of slight cerebral palsy mainly choreo-athetosis and ataxia learning difficulties, perceptual disorders and a syndrome of disturbed behaviour in children characterized by hyperactivity distractability emotional lability and inconsistency of intellectual function. As a large proportion of these children do not have any neuroanatomical sign of cerebral lesion nor have any evidence of an exogenous cerebral injury as an etiological factor the association of these disorders with a "brain damage" is not always justified. Various aspects of this controversial topic were discussed at one of the recent meetings of the international study group in Oxford and these reports and the group discussions are presented in this volume. The conference seems to have come to the conclusion that the concept of 'minimal brain damage' should be discarded. The symptoms should *instead be specified as far as possible* and for this purpose many of the papers contain valuable information about methodology and technique of investigation. It would have been of value to have included some critical comments by an experienced electroencephalographer as many of these children are sent for an EEG examination, which used as a routine method of diagnostic work-up seems to have very limited practical value. In general the present volume conforms well to the high standard one is used to from previous monograph of this series.

B. Hallström

Acta Paediatrica 53. 205–21... M y 1964

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Primary Aldosteronism due to an Adrenal Adenoma in a Three-year old Child

by B CAVELL, E. SANDEGÅRD and B. HÖKFELT

The clinical syndrome due to an aldosterone-secreting adenoma was first described some 8 years ago by Conn [9], who called it primary aldosteronism. It is characterized by potassium depletion, hypertension and increased aldosterone excretion. More than one hundred cases of primary aldosteronism have been reported, and it has become evident that Conn s syndrome is a symptomatic curable form of hypertension [10 14]. Early recognition of the condition is therefore important. In 70 % of the published cases of Conn s syndrome the diagnosis was made in patients ged 30–49 years [10]. The youngest patient so far reported was 15 years old [*0] That n aldosterone producing denoma can appear also quite early in life is illustrated in th following 3½ year-old patient in whom not only the classical symptoms were noted, but also stunted growth and possibly excessive hairiness.

Case report

History B M \ girl, born August 16, 1958, was first admitted in December 1961 at the age f 3 years and 4 months, because of increasing polyuria and polydipsia. She was number 3 f 3 siblings; her parents and siblings were healthy several f her relatives had diabetes mellitus. At birth the patient was normally developed (length 49 cm, weight 3000 g) and development during the first year of l f was normal. From l months of age she was ble to control urination during the day time but not at night From about 18 months of age nocturnal polyuria increased and the last few months before admission t hosp tal she voided once or twice an hour Apart from loss of appetit the patient had no symptoms. She was not abnormally tired, he showed no evidence f muscular weakness or tetany and did not complain of headache. During the last 6 months before hospitalization the mother had bserved that the patient had fine downy hair over the entire body but she did not know whether this increased lanugo had tended t progress. On perusal of the patient record f routine medical inspection it was found that she had ceased to grow from about 2½ years of age (Fig. 1).

Physical find ngs The patient wa small for her age height 83 cm (mean for her age 97 cm, range 90–105 cm), weight 10.5 kg (mean for her height 12.0 kg, range 10 5–14 kg). The musculature was reduced and the bdomen was tympanitic. There were no signs of Cushing syndrome (see Fig *) The body was covered with down, especially the arms and shoulders. There was no hypertrophy f the litoris. Physical examination of the heart revealed no pathologic signs.

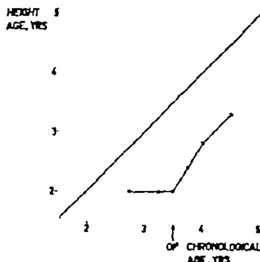


Fig. 1. Height age versus chronological age. Note growth arrest before operation.

The blood pressure in the left as well as in the right arm was 180/110 mm Hg. The femoral pulsation and the ocular fundi were normal. The tendon reflexes were somewhat accentuated, but Chvostek's sign and Trosseau's sign were negative.

Laboratory studies

Methods. Sodium and potassium in the serum and urine were determined by flame photometry (Eppendorf). Aldosterone and other steroid hormones were determined in 24-hour urinary specimens collected without preservative. Aldosterone was measured by a double-isotope derivative technique [17]; 17-ketosteroids by a modification of the Zimmermann reaction [18]; and 17-ketogenic steroids according to the method of Appleby *et al.* [1]. The steroid-hormone content of the adrenal adenoma (aldosteroma [4]) was assessed after incubation of tumor tissue with 7 H progesterone in a pH 7.4 saline phosphate buffer containing 200 mg glucose per 100 ml. The steroids were extracted, purified and separated principally according to Neher [19]; the positions of compounds were determined by scanning for radioactivity on a Baird Atomic strip scanner. Further characterization and quantitation was achieved by the application of UV absorption, soda fluorescence, tetrazolium blue, Porter-Silber and Zimmermann reactions. Urinary catecholamines were determined according to the fluorometric procedure of Euler & Lishajko [12]. Standard clinical methods were used for routine clinical tests.

Findings. Pertinent laboratory data are given in Table 1. Pre-operative studies showed hypokalemia and hypernatremia but normal serum bicarbonate. There was a markedly increased secretion of urine (Fig. 3) with a specific gravity varying between 1.003 and 1.006, the

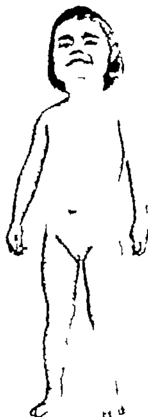


Fig. 2. Patient B. M. N. Physical appearance before operation.

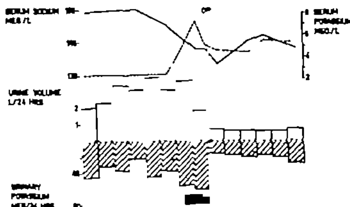


Fig. 3. Serum sodium (continuous line), serum potassium (interrupted line), urine volumes and urinary potassium. Urinary figures represent 5-day periods. Black area indicates administration of Aldactone (*00–150 mg/day).

latter value obtained following a concentration test Pitressin failed to increase the specific gravity of the urine. The urine was neutral or slightly alkaline and contained at most traces of protein Despite the low serum potassium the 4-hour urinary potassium varied between 33 and 59 mEq A strict balance study was prevented by the young age of the patient, her potassium int ke on n ordinary diet was, however estimated at about 35 mEq per day which would imply a slightly negative potassium balance (Fig 3) The existence of kaliopenia was supported by the electrocardiographi findings, namely T vector of low magnitude prolonged Q–T interval and occasional prominent U waves. The combined administration of extra potassium and an aldosterone antagonist (spironolactone) resulted in a positive potassium balance, as judged by both serum and urinary potassium values (Fig 3) and a marked fall in the serum sodium

Hb 13.8 g/100 ml. White blood cells 6200 per mm with a normal differential count Fasting blood sugar varied between 0 076 and 0 094 g/100 ml and the curve obtained for an oral glucose tolerance test was normal. The plasma calcium and phosphorus were normal (10 0 mg/100 ml and 4 4 mg/100 ml respectively) and alkaline phosphatase was within normal limits. The 24 hour excretion of aldosterone in the urine was 19–44 μg Although only limited data are available on the urinary excretion of steroids in early childhood, it is obvious that the urinary aldosterone was markedly elevated in our patient In two normal children, aged 18 months and 4 years, we have found 4 hour urinary aldosterone values of 2 and 5 μg respectively nd the upper limit of the normal adult range as determined by the procedure used in the present investigation, is about 15 μg/24 hours. The urinary excretion of 17 ketosteroid varied between 1.2 and 2 5 mg/*4 hours, which is slightly above normal, compared with a mean of 0 78 mg/*4 hours for children aged 1–5 years published by Prout & Snaith [22] who used the same procedure In children,

TABLE 1 *Serum and urine electrolytes and hormonal assays before and 5 weeks after operation (ordinary diet)*

	Before op.	After op.
Serum sodium (mEq/L)	145–160	132–142
Potassium (mEq/L)	2.4–2.5	4.8–5.6
Chlorid (mEq/L)	102–103	—
Standard bicarbonat (mEq/L)	29	23
Urine volume (ml/24 hrs)	2000–2700	450–725
Specific gravity		
at concentration test	1.006	1.021
after Pitressin	1.003	1.014
Protein	0–trace amounts	0
Sediment	0	0
Reaction	Neutral alkaline	Acid–alkaline
Sodium (mEq/24 hrs)	35–50	14–29
Potassium (mEq/24 hrs)	33–59	1–20
Aldosterone (μg/24 hrs)	19–44	0.1–0.6
17 ketosteroids (mg/24 hrs)	1.2–2.5	0.4–0.5
17-ketogenic steroids (mg/24 hrs)	2.4–6.1	1.5–1.9
Adrenaline (μg/24 hrs)	2.0	—
Noradrenaline (μg/24 hrs)	7.1	—

aged 3–4 years, we have found the urinary excretion of 17 ketogenic steroids to vary around 2–4 mg/24 hours. It is thus obvious that the excretion of 17-ketogenic steroids in our patient (2.4–6.1 mg/24 hours) was normal to slightly elevated. The urinary excretion of adrenaline was 2.1 and noradrenaline 7.1 μg/24 hours, which are normal values, and most likely excluded the possibility of a pheochromocytoma as the cause of hypertension.

X ray. The laboratory findings reported above made the diagnosis of primary aldosteronism likely and an intravenous renogram suggested the presence of an abnormal mass above the right kidney. The appearances of the sella turcica, heart and lungs were normal.

Treatment and postoperative course

During pretreatment with extra potassium (40 mEq/day) and spironolactone (Aldactone 200 mg/day) the serum potassium rose sharply to 7.2 mEq/L and the ECG showed signs of hyperkalemia. After reduction of extra potassium to 26 mEq/day and Aldactone to 150 mg/day the serum potassium promptly returned to normal. At operation the right adrenal was explored by the transabdominal route and a cortical adenoma 3 cm in diameter was found and removed. The tumor weighed 15 g; it was well encapsulated and its cut surface was golden-yellow. Microscopically the adenoma consisted of well-differentiated cells of benign structures, in some areas reminiscent of zona glomerulosa, in other areas, of the fasciculata and reticularis. There was no evidence of malignancy.

Analysis of incubated slices of the adenoma showed that the growth could produce not only aldosterone and corticosterone but also considerable quantities

TABLE _. *Steroid hormone content (µg/g tissue) following incubation of the aldosteroma.*

Cortisol	7 3
Cortisone	0.3
Aldosterone	0.3
Corticosterone	1 4
DOC	—
17-ketosteroids	3.3

of cortisol as well as compounds reacting with the Zimmermann reagent probably 17 ketosteroids (Table 2).

The postoperative course was uneventful. The concentrations of the blood electrolytes promptly became normal as did the output and the pH of the urine (Table 1) The blood pressure fell to 100/60 mm Hg almost immediately after the operation. The marked decrease in potassium excretion probably reflected a positive potassium balance (Fig 3) Urinary aldosterone fell to subnormal levels, while the urinary 17-ketosteroids and 17 ketogenic steroids were within the lower normal range (Table 1)

On examination 6 and 1 months after the removal of the adrenal tumor the patient felt well. Thirst and urine output were normal, she was growing at a normal rat (Fig 1) and her hirsutism had disappeared the blood pressure was normal and there was no electrolyte metabolic disturbance. Steroid hormone excretion wa normal 1 months after operation. aldosterone 2.4–3.4 µg/24 hours, 17 ketosteroids 0.5–0 6 mg/*4 hours and 17 ketogenic steroids 2.6–6.0 mg/*4 hours.

Discussion

The patient was brought to the doctor because of pronounced polyuria and polydipsia, and these symptoms in combination with retarded growth suggested a hypophysial tumor This possibility was however soon excluded by a normal roentgen appearance of the sella turcica and the occurrence of pitressin resistant polyuria Other symptoms of diagnostic value were the occurrence of hypertension and hypokalemia, combined with marked excretion of potassium in the urine These findings suggested Conn s syndrome and for this reason the patient was studied regarding the production of aldosterone.

In retrospect, the patient s history argues the overproduction of aldosterone for at least 18 months and the disease had probably resulted in considerable potassium deficiency Since potassium seems to be essential for normal growth, both in animals and in man [5 _5], the retardation of growth was probably due to kaliopenia. Retarded growth has also been observed in young patients with aldosteronism without adrenal tumor presenting all the clinical and biochemical manifestations typical of Conn s syndrome but with malignant hypertension [7 10]; this type of aldosteronism has been tentatively designated by Conn as "congenital aldosteronism" [10]. Furthermore growth retardation was found in two of the three patients recently reported by Bartter *et al* as a new syndrome characterized by hypokalemic alkalosis overproduction of aldosterone and hyperplasia of the juxtaglomerular complex, but with normal blood pressure [3 21]. Dwarfism was also present in another recently described entity with congenital hypokalemia of presumably renal origin without involvement of ldosterone· also these patients had normal blood pressure [8].

Hypertension is one of the earmarks of Conn's syndrome [10–14] and the presence of hypertension in young patients with hypokalemia and aldosteronism is thus of importance in differentiating aldosteroma from the kind of aldosteronism described by Bartter *et al* and referred to above [3]. The latter group possibly differs from Conn's syndrome also by having a rather low serum sodium in spite of normal capacity to conserve sodium on the kidney level. It is not clear why there is hypertension in Conn's syndrome but not in the patients described by Bartter *et al*. But the prompt normalisation of the blood pressure following the operation on our patient indicates a close interrelationship between the adenoma and hypertension.

It seems possible that the two above mentioned patients with congenital hypokalemia [8] are in some way related to the normotensive cases of aldosteronism described by Bartter *et al* [3]. In the former cases the authors ascribed the potassium wasting to a renal rather than an adrenal defect but no thorough study seems to have been performed with respect to aldosterone production. Thus in one of the patients no aldosterone measurements were reported in the other urinary aldosterone was determined once and then found to be slightly elevated this elevation was considered secondary to changes in electrolyte intake.

Before the operation the entire body of the patient showed slight lanugo hirsutism. This type of hairiness may occur normally but in our patient it was probably due to a slight overproduction of 17 ketosteroids by the tumor since incubation of the latter produced, among other things, substances which reacted with Zimmermann's reagent. On the other hand, both clinical and laboratory findings excluded the possibility of any substantially increased production of androgens.

On incubation the tumor also produced a considerable amount of cortisol. It is therefore possible that the tumor also secreted a certain amount of cortisol *in situ* but this secretion can hardly have been substantial, since the patient showed no clinical signs of Cushing's syndrome, and the excretion of 17 ketogenic steroids was, as a rule normal, though slightly elevated values were occasionally noted. If the production of cortisol by the adenoma *in situ* had been considerable it would have inhibited the secretion of ACTH by the hypophysis with consequent atrophy of the contralateral adrenal. This in turn would have caused adrenocortical insufficiency after removal of the adenoma that this did not occur is obvious from the fact that the patient did not require cortisone-substitution therapy after the operation.

The observation that an aldosteroma can produce a number of steroid hormones besides aldosterone on incubation *in vitro* is not new. Such production was first reported by Achee [19] and has since been found in several cases of aldosteroma [6–16]. *In vitro* studies are markedly influenced by the incubation medium used, as well as by other external factors. The demonstration of the synthesis of certain steroids in such assays therefore only shows that the tissue in question contains the enzyme systems necessary for such production but does not exclude the possibility that the tissue was also able *to* produce *other* hormones *in situ*, even

though no such hormones were demonstrable after incubation of the tissue. Neither do *in vitro* studies warrant any conclusions as to which hormone is produced in largest amounts by an adenoma *in situ*.

Histological examination of the adenoma showed varying types of cells but did not suggest that the adenoma had originated in any particular zone of the adrenal cortex. The occurrence of different types of cell appears to agree well with the capacity of the adenoma to synthesize a number of steroid hormones. If the adenoma had produced only aldosterone, one might have expected it to be built up exclusively of the type of cells present in the zona glomerulosa, since aldosterone is normally probably produced only in this zone [ ]. Moreover, the gross and microscopical appearance of the tumor in our patient resembled that seen in several cases of Conn's syndrome [10, 14]. The microscopical appearance did not suggest malignancy, an observation likewise in agreement with other published cases of aldosteroma. In this connection it might be relevant to recall that in childhood benign cortical tumors are less commonly the cause of Cushing's syndrome than are malignant neoplasms [24], even though Cushing's syndrome in association with adrenal virilism due to a presumed benign adrenocortical adenoma has been reported [23].

Decreased tolerance to carbohydrate in the form of glycosuria and a diabetic glucose tolerance curve [13] have been reported in several cases of primary aldosteronism. The decreased glucose tolerance can be corrected by administration of potassium and, in some cases also by spironolactone [11]. It is therefore remarkable that the patient did not show a decreased glucose tolerance despite kaliopenia and heredity for diabetes. Other symptoms ascribed to kaliopenia are muscular weakness and hyporeflexia or areflexia, sometimes flaccid pareses. Like children with hypokalemia and aldosteronism of "congenital type" [10], our patient had none of these symptoms.

Summary

A girl, aged 3½ years, was admitted to hospital because of polyuria, polydipsia and retarded growth. On examination she was found to have moderate hypertension and slight hirsutism. Laboratory studies revealed hypokalemia combined with potassium wasting in the urine, slight hypernatremia and high urinary aldosterone excretion. The urinary 17-ketosteroids were slightly elevated, whereas the 17-ketogenic steroids were normal or slightly elevated. Administration of spironolactone was followed by an increase of the serum potassium and a decrease of the serum sodium. An aldosteroma (Conn's syndrome) was diagnosed and successfully removed. On incubation the adrenal adenoma produced considerable quantities of aldosterone, cortisol and 17-ketosteroids.

Promptly after removal of the adenoma, the urinary aldosterone dropped to below normal, the blood electrolytes became normal, and polyuria, polydipsia and hypertension disappeared. When seen one year after operation the girl was perfectly healthy and growing at a normal rate.

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Cystinuria in Sweden

VIII A Case of Coeliac Disease Associated with Cystine-Lysinuria

by LEIF HAMBRÆUS and GEORG DE HEVESY

Introduction

Osborne & Mendel [21] were the first to demonstrate that the amino acid lysine was essential for growth in rats, and 27 years later Albanese and collaborators [3] showed it to be indispensable for growth in human beings. Several workers have investigated the 24 hour requirement of lysine in the human being For healthy young adults the latter has been reported to vary between 300 mg and 900 mg per day [8, 19 24] and for the growing infant it has been found to range between 90 mg and 200 mg per kg body weight per day [1 20]. In a study of healthy infants, Albanese *et al* [2] found that the nutritional value of many infant foods could be improved by small addition of lysine The part played by lysine in the nutrition of the human being has recently been extensively reviewed by Jansen [18].

Studies of the lysine balance in patients with increased urinary excretion of lysine such as homozygous cystinurics, whose daily output of lysine may be as high as g per day [28], have so far not been carried out to the best of the authors knowledge Some authors have observed that patients with homozygous cystinuria are slightly below average in height and have expressed the view that this might be due to deficient nutrition in infancy and childhood caused by the excessive excretion of lysine [4 9].

In this paper the case of an infant with excessive urinary excretion of lysine and cystine associated with clinical signs of malnutrition is reported. Bearing in mind the important part which lysine plays in protein metabolism, quantitative studies of the patient's intake and output of lysine were carried out

Dietary Experiments

During five periods, each of about 14 days duration, the patient was given different diets some being supplemented with varying doses of lysine

During the first period a diet was given (standard diet No. 1 providing about 850 calories and consisting of 120 g carbohydrates, 20 g fat and 35 g protein) which largely corresponded to the diet the patient had been given at home During the second period this diet was supplemented with oral lysine Thereafter the caloric and protein levels in the diet were increased (standard diet No. 2 providing about 1*00 calories and

consisting of 185 g carbohydrate 30 g fat and 40 g protein) This diet was gluten-free and was given during the third fourth and fifth periods, being supplemented with lysine during the fourth period.

Throughout each period samples of 24 hour feeds and 4 hour samples of urine and faeces were collected for analysis.

The samples of the feeds and faeces were homogenised in a Waring type blendor Thereafter these together with the samples of urine were stored (in a freezing-chamber) at −20°C. Prior to analysis the homogenised samples were hydrolysed in 6 N HCl at 100°C for 24 hours.

Analytical Methods

The following analytical methods were used:

(1) *Brand's test* [7]. This test was used for the demonstration of increased excretion of cystine.

(2) *Two-dimensional paper chromatography* Ascending paper chromatography in two dimensions according to the method described by Dent [11] was used for semi quantitative amino acid analyses, phenol water (500 g phenol + 1 5 ml aq dest) in ammonia atmosphere and lutidine-water (200 ml lutidine + 100 ml aq dest) in a ethylamine atmosphere being the first and second solvent respectively The paper chromatograms were allowed to dry in a stream of hot air for two hours and were then developed by dipping them momentarily in a 0 2% solution of ninhydrin in acetone They were allowed to dry and then heated in an oven at + 70°C for a few minutes.

(3) *High voltage paper electrophoresis* High voltage paper electrophoresis according to the method described by one of us [14], was used for separation and semi-quantitative studies of the basic amino acids excreted in the urine.

(4) *Ion exchange chromatography* A modified Spackman, Stein & Moore automatic amino acid analyser [27] for quantitative studies on the amino acids was used [15].

Case Report

K.E. a boy born in 1960 He was the third child, weighing 3600 g at birth. His mother's obstetric history was evidently normal. He was fed on breast milk alone for six weeks. Thereafter supplementary infant s food was given. He developed normally in weight. At seven months he weighed 7.5 kg.

When he was a few months old he developed a mild eczema on his face which was thought to be due to hypersensitivity to foodstuffs, particularly tomatoes. At the age of six months he was admitted to hospital with pneumonia on the right side His condition was critical because of threatened respiratory failure However he gradually improved and after a month he was discharged. After his discharge he did not gain sufficiently in weight despite unimpaired appetite There was nothing noteworthy about the faeces. The patient general condition deteriorated. He did not play with his siblings as he previously had done he cried easily and was irritable and querulous. He vomited periodically with resultant progressive loss of weight

At the age of fifteen months he was admitted to hospital for study of his failure to thrive. On admission he was found to be poorly nourished; his arms and legs were extremely thin and his abdomen was distended He weighed 7900 g. The liver and spleen were not enlarged. There was nothing noteworthy about the lymph glands, heart and lungs, and there were no signs of rickets.

The full blood count, and differential, the haemoglobin and the micro-E.S.R. were normal. The serum concentration of sodium and chlorides was increased, the increase being interpreted as due to dehydration. At later follow up examination the serum electrolytes were found to be normal. The phosphatase, transaminase and thymol turbidity values were within the normal range. Serum cholesterol was 124 mg% and the total serum lipids was 800 mg%. Micro-N.P.N. was 35 mg% Routine urinalysis did not reveal anything noteworthy

TABLE I *Findings on which a presumptive diagnosis of coeliac disease was based*

Examination	Findings
Physical examination	Emaciation; distended abdomen, Weight 7.5 kg at the age of 15 months
Glucose tolerance test	Linear curve
Xylose excretion test	Linear curve
Vitamin A tolerance test	Suboptimal response suggesting malabsorption
Fat content of faeces	4.0 g per day corresponding to 21 % of the fat ingested
Amylase and trypsin determination	Normal
Small intestine biopsy	Atrophic intestinal mucosa showing inflammatory changes

Brand's test [7] revealed the urinary excretion of cystine to be increased and two-dimensional paper chromatography showed that the pattern of urinary amino acid excretion deviated markedly from normal inasmuch as the cystine and lysine levels were abnormally high.

The patient was put on a standard diet which largely corresponded to the feeds he had received at home but was insufficient for an infant of his age (standard diet No 1). After two weeks supplementary lysine (2.5 g lysine monohydrochloride) was given by mouth in addition. This diet did not result in weight gain or improvement in his general condition. Towards the end of this trial period, while still in hospital, the patient developed nosocomial enteritis and parotitis.

The glucose tolerance test (2 g glucose per kg body weight) and xylose excretion test [23] produced a nearly linear curve. This together with the result of the vitamin A tolerance test [22] and the demonstration of 20 % of the ingested fat in faeces suggested malabsorption. Small intestine biopsy showed signs of atrophy and inflammatory changes. On the basis of these findings together with the clinical picture a presumptive diagnosis of coeliac disease was made (Table I)

For further investigation the patient was put on a gluten free diet. The protein level in the diet was slightly increased (from 35 g to 40 g per day), and to meet more adequately the nutritional requirement of the patient the number of calories was increased to 1200 per day (standard diet No 2). He was on this diet for three weeks (period No 3) and thereafter it was supplemented with lysine, giving 3.5 g lysine monohydrochloride per day (period No 4). The weight curve (Fig. 1) showed that the standard diet No 2 alone resulted in weight gain. His general condition improved markedly; he had gained 1.4 kg in weight over six weeks and was well covered. There was also a marked improvement in his mental condition. The laboratory findings confirmed the clinical improvement. The values of the xylose excretion test had reverted to normal and the results of the vitamin A tolerance test were almost normal.

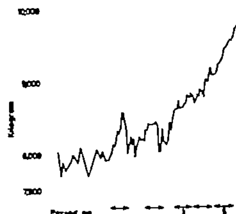


Fig. 1 Weight curve showing the patient's gain in weight during different periods

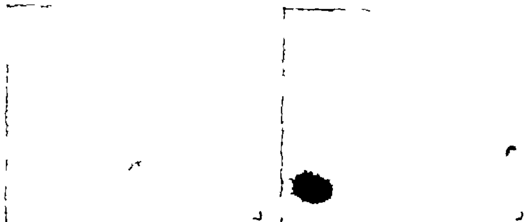


Fig. — P per chromatograms of samples of urine. Two-dimensional paper chromatography according t Dent [11] on a specimen of urine from a healthy child of the same age as the patient (on the left) and from the patient himself (on the right).

However investigation of the pattern of urinary amino acid excretion did not reveal any improvement and the patient continued to excrete abnormal quantities of lysine Supplementary lysine did not appear to have played a part in the patient s improvement as he continued to gain weight fter it withdrawal (period No. 5)

At the follow up examination four months later when the patient was two years old, he appeared t be normally developed. His ɔeral condition was good, he was well ep ered and the development of his muscles corresponded t his age According to the informat on given by his mother he did not show any psychological abnormalities and had a good appetit He had progressively gained in weight weighing then 11 4 kg

Results

Fig 2 shows two paper chromatograms the one on the left illustrates the findings in a sample of urine obtained from a healthy child of the same age as the patient, and that on the right those in a sample of urine obtained from the patient. In the latter chromatogram two spots are seen which are not visible in the former The position of the dense spot in the lower left corner corresponds to that of the basic amino acids The position of the small dense spot to the right corresponds to that of cystine the latter for technical reasons, being demonstrated in the form of cysteic acid [10]

Fig 3 shows the electropherogram of specimens of urine obtained from the patient (specimens Nos. 1 and 7) of 1 specimen from each of his parents (specimens Nos 2 and 3) and 1 specimen from each of his two brothers (specimens Nos 5 and 6) Specimen No 4 is derived from a standard mixture of the amino acids arginine lysine and ornithine which are visible in the form of dense discrete spots. On the *conventional paper chromatogram* these amino acids do not appear as discrete spots. It is seen that the lysine concentration in the patient s urine is markedly raised, the values of the concentration of the basic amino acids lysine arginine and ornithine in the specimens of urine obtained from his relatives being within the normal range

Analysis of samples of urine obtained from a further fourteen relatives of the patient did not reveal an abnormal pattern

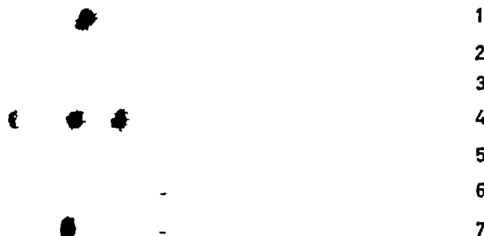


Fig. 1. *Electropherogram of samples of urine.* High voltage paper electrophoresis according to Hambraeus [14] on two specimens of urine from the patient (Nos. 1 and 7), one specimen from each of his parents (Nos. 2 and 3), and each of his two brothers (Nos. 5 and 6). A standard mixture of the amino acids arginine (ARG), lysine (LYS), and ornithine (ORN) is included as comparison (No. 4).

of urinary amino acid excretion in any individual case.

Quantitative analysis of the amino acids in the specimen of urine obtained from the patient on admission, using an automatic amino acid analyser, revealed the presence of grossly abnormal quantities of lysine (230 mg per day or 1840 mg per g creatinine) and an increased output of cystine (48 mg per day or 370 mg per g creatinine) arginine (5 mg per day or 37 mg per g creatinine) and ornithine (18 mg per day or 141 mg per g creatinine). Six month later analyses of the urinary specimen obtained from the patient at the follow-up examination showed that the output of arginine had increased (23 mg per day or 100 mg per g creatinine) the excretion of the other three amino acid being practically unchanged (Table 2).

Table 3 shows the results of the quantitative studies of the lysine intake and output in the different trial periods. It is seen that the urinary excretion of lysine per day was the same while the patient was on the standard diets 1 and 2 despite the fact that the lysine content of the latter was about 30% higher than that of the former. When these diets were supplemented with lysine the lysine excretion per day increased, about 10% of the supplementary lysine being lost in the urine.

The concentration of lysine in the faeces varied but the variation did not seem to be related to the total quantity of lysine ingested.

TABLE 2 *Quantitative determinations of the amino acids cystine lysine arginine and ornithine in specimens of the patient's urine obtained on admission and at follow-up examination*

	Urinary excretion			
	On admission		At follow-up	
Amino acid	mg per day	mg per g creatinine	mg per day	mg per g creatinine
Cystine	48	380	43	400
Lysine	250	1940	305	1390
Arginine	5	40	22	100
Ornithine	*18*	*140*	*20*	*80*

The amount of creatinine excreted in the urine per day was roughly the same in the different trial periods. This observation confirmed that the 24 hours samples collected were in fact the true total 24 hour urinary output

Discussion

The diagnosis of coeliac disease was based on.

(1) the almost linear curves obtained at the xylose excretion test and glucose tolerance test;

(2) the impaired absorption of fat in the gut

(3) the results of the vitamin A tolerance test,

(4) the findings on small intestine biopsy (Table 1)

There are two feasible explanations of the patient's amino aciduria. Firstly it may have been caused by the coeliac disease *per se* Secondly two different diseases may have co-existed, viz. coeliac disease and amino aciduria of another origin.

The co-existence of amino aciduria and coeliac disease has been observed by several workers Bickel & Souchon (6) carried out semi-quantitative studies of the pattern of urinary amino acid excretion associated with steatorrhea and coeliac disease and found that a mild form of generalised amino aciduria was usually present which resembled that associated with

TABLE 3 *Quantitative studies of lysine intake and output.*

Trial Period No.	1		3	4	5
Standard Diet No.	*I*	*I*	*II*	*II*	II
Protein content (g per day)	35	35	40	40	40
Urinary volum (ml per day)	600	770	240	280	300
Creatinine excreted (mg per day)	180	160	—	160	180
Lysine intake (mg per day)					
In food	1030	1970	2580	2980	2750
Supplementary	—	2000	—	2800	—
Total	1030	3970	4080	5780	2750
Lysine output (mg per day)					
In urine	210	410	240	420	180
In faeces	—	240	410	320	380
Total		650	650	740	570

N data available

hepatic disease This observation was con firmed by Snyderman [25] and Ghadimi & Shwachman [13].

The pattern of urinary amino acid excretion observed in the case presented differed from that which has been reported to co-exist with coeliac disease inasmuch as the excretion of lysine cystine arginine and ornithine only was increased. Moreover the defect was quite severe because ex cessive quantities of lysine were excreted We are therefore inclined to believe that two different diseases co-existed in the case reported, viz. coeliac disease and a specific amino aciduria similar to that of classical cystinuria.

The patient appeared to have main tained a positive lysine balance despite the lysine losses as the lysine requirement of a child of the patient s age has been re ported to be 1 g– g per day [1 ?6]. However it should be pointed out that no reliable method for the determination of lysine loss in the gut due to bacterial catabolism of lysine is s yet available According to Milne *et al* [5 ?0] bacterial catabolism of lysine occurs to a consider able degree in healthy individuals and to a marked degree in patients with cystinuria For these reasons no definite conclusions can be drawn from the results of the pre sent study However the findings sug gested that the quantities of lysine ex creted in the urine were not sufficiently large to cause lysine deficiency The clinical course of the disease substantiated this assumption.

Although the 4 hour excretion of crea tinine is lower in infancy and in childhood than in adult life it was cons dered to be of interest t determine the lysine creatinine ratio in the case presented On comparing the values obtained with those published by Harris *et al* (16 1?] and Fow ler *et al.* [1] it was found that they were abnormally high and of the same magni tude as those obtained in cases of classical cystinuria, even following correction for age variation in creatinine excretion.

The cystine/creatinine ratio and argi nine/creatinine ratio also appeared to be abnormal but were within the range of the average values given for homozygotes and heterozygotes with cystinuria [17] As no data are available on the pattern of urinary amino acid excretion in cystinuric patients of the same age as our patient nothing definite can be said about the type of his cystinuria, i.e whether it was heterozygous or homozygous.

Summary

A case of failure to thrive associated with excessive excretion of cystine and lysine in a male infant of fifteen months is described. Quantitative studies of the patient s lysine intake and output sug gested that the increased lysine excretion *per se* was not the primary cause of the malnutrition. On the basi of the clinical and laboratory findings a presumptive diagnosis of coeliac disease associated with classical cystinuria was made

Ackn wledgements

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The Cardiovascular Component of Congenital Diaphragmatic Hernia (Presentation of Three Cases)

by PABLO JIMENEZ BRUNDELET and JOHANNA SUCKSDORFF

Congenital diaphragmatic hernia (usually left-sided) associated with hypoplasia of the lung situated on the same side as the hernia (usually the left one) constitutes a very well-known entity [1 7] What appears to be less well-known, is that the heart and the great vessels of these patients present a characteristic picture consisting of a variable hypoplasia of the left heart [1] a variable hypertrophy of the right heart plus an enlarged ductus arteriosus. The description of the following three cases in which the 'triad' left-sided diaphragmatic hernia pulmonary hypoplasia and the above-mentioned abnormalities of the heart and great vessels were observed at autopsy will emphasize in detail the cardiovascular anomalies.

Case 1

The first case was the second child of 28 years old mother The first child is healthy Emesis gravidarum was present during early pregnancy Six weeks before delivery acut hydramnion developed needing t be punctured once t remove the excess of fluid. It was term delivery in cephalic presentation and the amniotic fluid was clear During delivery the heart sounds became slow (90 to 120 per minute); a prolapsed umbilical cord was found, the vacuum extractor was used at once but the heart stopped during this procedure and a dead male infant measuring 56 cm in length, and weighing 4000 g, was delivered.

Necropsy On *external examination* there was a moderat degree of cyanosis f nail beds and lips. On *internal examination* the abnormal findings were mainly present in the thoracic cavity The left hemidiaphragm presented a large opening (bout 4 cm in diameter) one side of it being directly formed by the posterior costal wall (Bochdalek hernia) Through this diaphragmatic opening abdominal organs had herniated int the left pleural cavity: the stomach, the jejunum, the ileum, the caecum and part f the ascending colon, the spleen and part f the left lobe f the liver were present in the left pleural sac N hernial sac was found. The left lung (Fig 1) wa very small, clearly hypoplastic and was compressed against the mediastinum which wa itself shifted towards the right hemi-thorax. The right lung was moderately smaller than normal due t compression against the chest wall by the mediastinal shift The combined w ight of both lungs was 10 g after formalin fixation. After opening the pericardium, the heart (weight 23 g) showed a moderately enlarged (hypertrophied and dilated) right ventricle (thickness 3 mm) and right atrium (Fig. 2); making a striking contrast the left ventricle (thickness mm) and the left atrium were very small, compared with

[1] The words left heart and right heart are used here in broad sense including the afferent and efferent blood vessels.

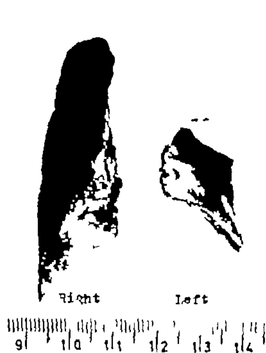


Fig. 1 Shows the marked hypoplasia of the left lung (only a small part of it, indicated, by the broken line was taken for histological examination).



Fig. 2. Shows the right ventricular hypertrophy and dilatation, and the opened ductus arteriosus.

what they should be at birth in a normal infant at term; they were therefore clearly hypoplastic (Fig. 3); the pulmonary veins, anatomically normally situated were also very small, and so were the ascending aorta and the aortic arch. The ductus arteriosus was not only patent but enlarged, its calibre being slightly wider than the calibre of the left pulmonary artery and about the same as the calibre of the right pulmonary artery at their point of origin from the common pulmonary trunk which was itself enlarged. Due to the hypoplasia of the aortic arch, the main pulmonary arterial trunk was giving the impression of being, through the ductus arteriosus, in direct continuity with the descending aorta (Figs. 2 and 3). The foramen ovale was anatomically patent. No other intra-cardiac abnormality was present. The remaining organs showed no striking abnormalities of interest.

Case 9

The patient was a two days old male infant. He was the third child of a 23 years old mother. The pregnancy and delivery were normal, except for excess of amniotic fluid noticed at delivery time. Birth weight 3180 g. The infant was noticed to be cyanotic since birth; his breathing was intermittent, and the heart sounds were heard displaced in the right hemithorax. A radiography of the chest confirmed the presence of a huge left-sided diaphragmatic hernia with a marked mediastinal shift towards the right side. An operation performed on the same day revealed a diaphragmatic defect in which the posterior part was directly formed by the posterior costal wall (Bochdalek hernia), and through which three fingers could be inserted. Through this opening, the stomach, the spleen, the left lobe of the liver, a large part of the small intestine and part of the colon were herniated into the left pleural cavity

without any obvious hernial sac present The organs were replaced back into the bdomen, and the diaphragmatic opening was closed with a suture The left lung was noticed t be small, but it seemed t re-expand partially at the end of the operation. A stormy post operative course followed: the breathing was intermittent; air was found first in the left pleural sac, and later on, in the right one needing t be aspirated first with a syringe and a needle, and later on, by inserting rubber t be int each pleural cavity and applying a continuous suction with nega tive pressure aspiration. In spite f all these measures, the patient died the day after the operation.

Necropsy On *external exam nation* cyanosis f nail beds and lips was present; a recent surgical incision was present in the left hypochondrium, and a rubber t be was in serted in each pleural cavity On *internal examination*, the interesting findings were mainly in the thoracic cavity The left lung was small, clearly hypoplastic although slightly less than in the first case and was not filling the left pleural sac even after inflation with formalin. The left pleural space was partly occupied by blood clots. The mediastinum was still moderately shif ted towards the right side and the right lung was compressed against the chest wall and partially collapsed. Inflation with for malin prevented t investigat the weight f the lungs. The diaphragmatic defect was surgically repaired and in good condition. The heart (weight 17 g) showed moderately enlarged right ventricle (thickness 3 mm) and right atrium, although slightly less than in the first case, making a moderat contrast the left heart was moderately hypoplastic; small left atrium, small left ventricle (thick ness 3 mm), small pulmonary veins, small ascending aorta and small aortic arch; the ductus arteriosus was not only patent but also enlarged, its diameter being of the same size as the diameter f the right and left pulmonary arteries at their point of origin from the common pulmonary trunk. The common pulmonary trunk was itself wider than normal. The foramen ovale was anatomically patent. No other intracardiac abnormality was present. In the peritoneal sac, the bdominal organs were showing no evidence f infarction, but the stomach presented a curious tri lobulated appearance alongside the greater curvature (probably the result f partial herniation through the diaphragmatic opening), and the part of the left lobe f the liver which was previously herniated, had a somewhat deformed shape The remaining organs showed no obvious abnormality



Fig 3. Shows the small left atrium, the small left ventricle the small ascending aorta, and the enlarged ductus arteriosus.

Case 3

The third case was the first child (weighing 3150 g and measuring 48 5 cm in length) f a 20 years old mother The pregnancy and delivery were normal. The amount of amniotic fluid was reported to have been about normal. When a few hours old the infant was noticed to have respiratory difficulties,

and on medical examination a left-sided dia phragmatic hernia was diagnosed. An opera tion performed by abdominal route revealed a Bochdalek hernia with an opening admit ting about two fingers. The posterior border of the hernia was partly formed by the costal wall and partly by the left kidney which was very highly situated, having its upper half in the thoracic cavity The stomach, the entire small intestine and part f the colon were present in the left pleural cavity These organs were easily pulled back into the abdomen but the diaphragmatic opening proved to be very difficult to close due to the size of the hole and the degree of stretching of the left hemi-diaphragm when closure was attempted. However with four se parated linen stitches (two using the 12th rib and two using the anterior peri renal tissue) the diaphragmatic defect was finally successfully closed. A stormy postoperative course followed: the following day a tension pneumothorax occurred producing a marked mediastinal shift towards the right side and in spite of the insertion of a rubber tube in the l ft pleural cavity and continuous aspira tion, two days after the operation the heart stopped and the patient died.

Necropsy On *external exam nation*, cyano si of lips and nail beds was present, as well as a recent abdominal incision, and a rubber tube inserted in the left pleural sac. On *internal examination* the findings of im portance were in the thoracic cavity There was a dehiscence f the two more externally situated linen stitches, with a resulting partial recurrence of the diaphragmatic opening (about 1 cm in diameter) through which the left splenic part of the colon and part of the transverse and descending colon had herniated. This herniated part of the colon had become irreducible by meconial accum lation inside it. The two more me dially situated linen stitches (uniting the diaphragm to the anterior peri-renal tissue) were in good condition. The left kidney was situated higher than normal its upper half being in the left thoracic cavity The left adrenal was also situated in the thoracic cavity The left lung was moderately small, hypoplastic (but not as much as in the two previous cases) appearing "collapsed" against the mediastinum, and remaining small even after formalin inflation. The mediastinum was shifted towards the right side *The right lung was slightly* smaller than normal, being moderately compressed between the chest wall and the displaced mediastinum. Inflation with formalin pre vented to investigate the weight of the lungs. After opening the pericardium, the heart appeared small (weight 14.5 g), showing a mild enlargement of the right atrium and right ventricle (thickness 3 mm). The left atrium, left ventricle (thickness 4 mm), and pulmonary veins were slightly smaller than normal. The ascending orta and the aortic arch were slightly small and narrower than the main pulmonary arterial trunk which was itself slightly enlarged. The ductus ar teriosus was enlarged of about the same size as the left or right pulmonary artery at their point of origin from the common pul monary arterial trunk. It has to be em phasized that the anomalies in this case were of mild degree and that the more apparent ones were the smaller left chambers.

Discussion

As is apparent from the above descrip tions the three cases presented respec tively a marked a moderate and a mild degree of the same condition. They had in common not only a *left-sided diaphrag matic hernia* (Bochdalek type) of import ance (several or parts of several abdominal organs being present in the thorax, and occupying most of the left pleural cavity), a *hypoplasia of the left lung* of marked degree a mediastinal shift towards the right side producing a moderate degree of com pression hypoplasia of the right lung and an identical group of cardiovascular ano malies. *It was not always easy* to decide if the slightly small right lung was the result of only collapse due to compression

or if there was associated some minor degree of hypoplasia as well. Inflation of the lungs with formalin, performed in the last two cases, produced a limited re-expansion of the left lung which was still very hypoplastic after the inflation, the right lung, on the contrary seemed to re-expand to an approximately almost normal volume (mainly in the third case) giving the impression that the compression-collapse played a substantial role and that if hypoplasia was present as well, it appeared rather mild. On account of this slightly undecided and possibly variable factor we use the word compression hypoplasia to refer to this possible mixed state of the right lung The cardiovascular anomalies consisted in the three cases of a relative hypoplasia of the left heart that is, a small left ventricle a small left atrium, small pulmonary veins, small ascending aorta and a small aortic arch were present; the right heart on the contrary showed a relative enlargement (mixture of hypertrophy and dilatation) of the right atrium and ventricle the common pulmonary arterial trunk was also wider than normal, finally an enlarged ductus arteriosus was present with a lumen of about the same size or wider than the size of the lumen of the right or left pulmonary artery at their point of origin from the common pulmonary arterial trunk. The heart weight was in the first two cases within the lower part of the normal weight range (_3 8 g $\pm$5 3 for the first case 20 7 g$\pm$5.3 for the second case) probably because the right heart hypertrophy was relatively compensating the decrease in weight due to the left heart hypoplasia, but in the third case the heart weight was almost 1 gram below the normal weight range (*0.7 g$\pm$5.3) and this in spite of the mild right heart hypertrophy which was not enough to compensate the decrease in weight due to the left heart hypoplasia [3].

The pathogenesis of the cardiovascular component of these multiple congenital abnormalities appears to be partly the result of the marked hypoplasia of the left lung and of the variable compression hypoplasia of the right lung The resulting reduction of the pulmonary volume prevents the normal development of the pulmonary vascular bed, the amount of blood circulating through the lungs being therefore small. Due to the small pulmonary vascular capacity the blood circulating through the pulmonary artery not only finds a route of escape via the ductus arteriosus which is open during intrauterine life but also produces an enlargement of its lumen and prevents its normal closure Also due to the smaller amount of blood circulating through the reduced pulmonary vascular bed, the pulmonary veins are themselves less developed, hypoplastic less blood is reaching the left atrium and therefore also the left ventricle explaining why these chambers of the left heart are less developed than they should normally be in other words why they are hypoplastic There is, however an additional factor which we believe is also responsible at least partly in preventing the normal development of the left heart That is the direct compression of the left side of the heart by the abdominal organs which are present in the left pleural cavity If these herniated organs are able by their presence to prevent the normal development of the left lung there is no reason why they should not prevent up to some

extent, the normal development of the left heart which normally occupies a place partially situated in the left hemi-thorax

The right heart hypertrophy is probably best explained by the fact that most of the blood which should normally pass into the left heart via the foramen ovale can no longer do so not because of the hypothetic closure of the foramen ovale which is not present in these cases but because of the compression of the left heart which prevents the left chambers to expand sufficiently Most of the blood reaching the right atrium passes therefore into the right ventricle which receives more blood than usual and which has also to send more blood into the common pulmonary arterial trunk The result is that a very small amount of blood circulates through the left heart and the lesser circulation, while a larger amount of blood circulates through the right heart The left heart hypoplasia and its accompanying circulating hypovolemia are probably simultaneous phenomena but it appears that it is the circulating hypervolemia in the right heart which is responsible for the right heart hypertrophy This explains also why some cases (Case 1) show a marked right heart hypertrophy while others (Case 3) show only a minimal right heart hypertrophy depending on the reduction in volume of the left chambers the more hypoplastic the left heart is the more hypertrophied will the right heart be and vice versa, a mild left heart hypoplasia will be accompanied by a minimal right heart hypertrophy The exact anatomical situation of the cardiovascular system in these cases is well summarized by saying that there is a left heart hypoplasia a right heart hypertrophy and an enlarged ductus arteriosus.

If we now replace the cardiovascular anomalies within the pathogenesis of the other congenital abnormalities, it appears that they may be considered as a sequence of events in which the primum movens is the diaphragmatic defect It is the diaphragmatic opening which allows the presence of abdominal organs in the left pleural cavity preventing the development of the left lung (which remains very hypoplastic) compressing the left side of the heart, shifting the mediastinum towards the right side causing a mild to moderate compression hypoplasia of the right lung, and producing as result of all these changes a hypoplasia of the left heart first, a secondary hypertrophy of the right heart and a persistence of an enlarged ductus arteriosus as a derivation route

These three cases presented what has been called a hypoplastic left heart syndrome (*) Although offering in many cases some common findings this syndrome shows on careful examination intra-cardiac anatomical differences which are probably due to different causal mechanisms. Therefore, even if the grouping together of several pathological entities under the common name of hypoplastic left heart syndrome is at first sight very attractive it is nevertheless necessary to divide them in sub-groups according to the associated anomalies and also according to the etio-pathogenesis in cause. Furthermore, the right heart hypertrophy is also a part of the typical heart of congenital diaphragmatic hernia even if it is secondary to the left heart hypoplasia and perhaps not always so striking In the three cases reported here, the characteristic grouping of

cardiovascular anomalies is the result of a common cause and mechanism and we believe that any left-sided diaphragmatic hernia of importance enough to produce a marked hypoplasia of the left lung and a mild compression hypoplasia of the right lung will also present the described cardiovascular findings which are partly secondary to the lung changes and partly secondary to the direct compression of the left heart.

To the best of our knowledge the past literature about this condition is rather scanty. The first case appears to have been published by Raeburn in 1951 and was thought to be produced by a premature closure of the foramen ovale due to pressure of the diaphragmatic hernia [5]. The association of this characteristic heart with congenital diaphragmatic hernia has also been mentioned in an excellent paper [4] studying the general problem of the hypoplastic left heart syndrome. Unfortunately no details are given in this paper about the cases and no mention is made about the state of the lungs. Finally in another previous paper [6] in about three-quarters of patients with congenital diaphragmatic hernia (22 cases) the autopsy revealed definite (10 cases) or questionable (4 cases) cardiovascular malformations which were of many different types without any particular pattern. Few details are given about these cardiopathies and no details are given about the state of the lungs or the importance of the diaphragmatic hernia. Without denying the possibility of congenital diaphragmatic hernia being associated with more complex types of cardiac defect like the ones mentioned in the literature we believe that even in the absence of these complex cardiac defects, there is a typical heart associated with left-sided diaphragmatic hernia of importance.

It is not unduly surprising that the characteristic cardiovascular component of congenital diaphragmatic hernia has not been more frequently reported, because in the cases successfully operated, if the pericardium has not been opened, the heart configuration is not visible at operation. The only noticed abnormality is the small left lung which is very well known to "grow" spontaneously up to its normal size during the following weeks, provided the left pleural cavity is kept free from air and fluid [7]. In these cases, when the lungs grow developing their vascular bed, and when the previously existing compression of the left heart has disappeared, there is the possibility for the hypoplastic left heart to grow, that is to develop up to its normal volume; this also includes the possibility for the ascending aorta and the aortic arch to develop, and for the enlarged ductus arteriosus to close down when need for its patency does no longer exist. In this respect, only the cardiologic study of the patients successfully operated on would permit to know more about the evolution of the hypoplastic left heart and also about the evolution of the enlarged ductus arteriosus. It is more surprising that from autopsy material more cases have not been reported. We think that some cases (like our Case 3) are showing only a mild degree of the cardiovascular component and there is the possibility for such cases to pass unrecognized unless the pathologist is especially aware of this condition.

This typical "heart associated with congenital diaphragmatic hernia of im

portance is not explained by any of the three usually mentioned theories (premature closure of the foramen ovale enlarged ductus arteriosus, true malformation) Patency of the foramen ovale in our three cases excludes the first cause Although an enlarged ductus arteriosus was present in all three cases this one was not the cause of the cardiovascular anomalies, but the consequence of the pulmonary hypoplasia therefore not a primary phenomenon but a secondary one Finally it does not appear to be a true malformation "per se because even if not directly proved yet, we are inclined to believe (as already mentioned above) that the hypoplastic left heart will probably develop after the surgical repair of the diaphragmatic hernia That would not happen to a true malformation, and this points out to the lung and heart anomalies as being only secondary phenomena to a diaphragmatic hernia of importance They could be considered as examples of deficient development due to a primary lack of space and susceptible of a delayed development when the normal space for it is restored. In this syndrome the diaphragmatic opening constitutes the primary abnormality or true malformation.

Finally the presence of an excess of amniotic fluid at the end of the pregnancy in two out of the three cases presented here, is to be emphasized. It has been found to occur in cases of congenital diaphragmatic hernia [6] A disturbance in the normal circulation of the amniotic fluid through the fetal alimentary tract (? obstructive factor) is perhaps responsible for it.

Summary

Three autopsy cases with the 'triad left-sided diaphragmatic hernia, pulmonary hypoplasia and left heart hypoplasia-right heart hypertrophy plus enlarged ductus arteriosus are presented. The probable etiology and pathogenesis of the cardiovascular component of this pathological entity are given, and it is believed that even in the absence of any other associated congenital abnormalities (intracardiac or extracardiac), any left-sided congenital diaphragmatic hernia of importance enough to produce pulmonary hypoplasia, will be associated with a typical "heart" showing a variable (from mild to marked) hypoplasia of the left atrium, left ventricle pulmonary veins, ascending aorta and aortic arch contrasting with a variable enlargement of the right atrium and right ventricle an enlarged common pulmonary arterial trunk and finally an enlarged ductus arteriosus as a derivation route.

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Infections and Staphylococci in Maternity Wards II

by B CEDERGREN R LAGERCRANTZ and B NYSTRÖM

In an earlier investigation hospital infections probably caused by Staphylococcus aureus (S aureus) were registered in 11 % of mothers and infants in the maternity wards of Karolinska Hospital [1]. S aureus were cultured from 82 % of the infants, 64 % of the mothers and 54 % of the staff Phagegroup III strains (particularly those of phage type 47) seemed to cause infection more often than other strains (including strains of phage-type 80/81)

The frequency of probable hospital infections and of carriers was considered relatively high In an attempt to lower this, ordinary soap for hand washing for mothers and staff was replaced with pHisoHex (Winthrop) as this has been reported a valuable hand desinfectant [2 3] To evaluate the effect of pHisoHex it was introduced only in the delivery rooms and in one ward while ordinary soap for hand washing was still used in the other wards. The result of a study of rates of infections and carrier states is presented in this paper

Material, methods and definitions

pHisoHex *instead of ordinary soap for hand washing for mothers and staff was used in the delivery rooms and in Ward no I for two weeks before and during the investigation, which lasted one month. In the other two wards ordinary soap was used as before No other changes in the ward routine were made since the earlier investigations.*

Infections in mothers and children were registered during their stay at hospital. Infected patients were isolated. On discharge the mothers received questionnaires concerning infections in mothers, babies or other members of their families. These questionnaires should be returned one month after delivery Missing or incomplete answers were completed by telephone call.

As probable hospital infections were registered:

In infants: pemphigus neonatorum, conjunctivitis, sties, umbilical infections and mastitis,

In mothers and infants upper respiratory tract infections with onset in hospital or during the first three days after discharge.

In mothers: mastitis (fever swelling and pain in the breast) and in other members of the family furunculosis, skin abscesses and sties.

Cases where it could not be decided whether conjunctivitis and skin rashes were of infectious or toxic-allergic origin were registered as possible hospital infections, as were cases with breast symptoms without fever (pain with or without rhagades).

During the first second and fourth week nose swabs were taken from all members of

TABLE 1 *Incidence of probable and possible hospital infections in mothers and children first month after delivery Number within brackets percent*

Year	1959	1960	1961	1959	1960	1961
N of individuals	238	172	270	238	172	270
Site of infection	Probable hospital infection			Possible hospital infection		
Child						
upper resp. tract.	1	—		—	1	—
eye	8	3	13	2	4	4
skin	10	6	7	4	3	—
breast	1	—	—	—	—	—
Mother						
upper resp. tract.	3	1	—	2	—	—
breast	8	9	11	7	16	8
Sum	31(13)	19(11)	33(12)	15(6)	24(14)	12(4)

the staff Nose swabs from mothers were taken during their first day in hospital and the day before discharge (as rule five or six days after delivery) On this latter day swabs were also taken from the mothers nipples and from the infants noses and groins

S abs were kept in moist serum t bes until cultured on blood agar plates. These were incubated for 18 hours at 37°C They were then read, and suspected St phylococcus colonies were tested with tube coagulase test Coagulase positive St phylococci were registered as S aureus. They were tested against the usual antibiotics according t the paper disc method for quantitative sensitivity tests described by Ericsson *et al* [4]. Strains were registered as sensitive f they were inhibited by the following concentrations:sulphonamide 10 mg %, penicillin 2 IU/ml, tetracycline 4 mcg/ml, chloramphenicol 16 mcg/ml and erythromycine 6 mcg/ml. Phage t ping f S aureus was made t the Swedish State Bacteriological Laboratory

Results

I fections T ble 1 shows the number of infected individuals in this and our earlier investigations. (Reports were available in 96 % of all families) Probable hospital infections were in this investigation observed in 14 % per cent of patients in Ward no 1 and in 10 % of those in the other two wards. There is no statistically significant difference between these values

In six cases the symptoms first appeared in hospital (within the first week after delivery), in twelve during the second or third week after delivery and in the remaining cases in the fourth week. Cases of pemphigus neonatorum were mild, only one child had to be readmitted to hospital. In this case the strain of S. aureus isolated from a blister was identical as to phage type and antibiogram to the strain isolated from the child s nose during its first stay in hospital. Maternal mastitis was more serious, as 4 cases required incision.

S ureus carriers The frequency of nose carriers of S ureus was as high in this investigation as in the earlier ones (Fig 1 and 2) and no significant difference wa found in carrier frequency between Ward no I where pHisoHex was used, and the other two wards.

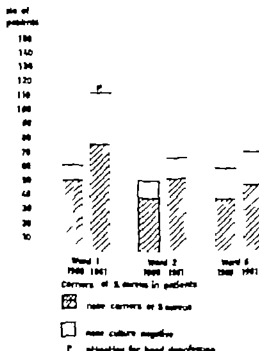


Fig 1 Carriers of S aureus in patient of the maternity ward in Karolinska Hospital.

Relation between S aureus and hospital infection S aureus was isolated in 19 of 32 individuals with probable hospital infection and in 143 of 204 without S aureus was not isolated in a statistically significant higher frequency in infected than in healthy patients. Most of the strains that could be typed belonged to phage group I or III. Strains of phage type 80/81 occurred in 16.5 % of infected patients and in 7.5 % of not infected patients. This difference is statistically significant on the level $0.05 < p > 0.01$ No other phage group was represented in a statistically significant higher frequency among infected than among uninfected patients. Thirty-seven per cent of the isolated strains of S aureus were not typeable. Strains from different sites are compared in Table 3 Table 4 relates the carrier state in the mothers at admittance with the occurrence of hospital infection. In 8 cases of probable hospital infection a strain could be isolated from the mother's nose at admittance and on discharge In five of these a change of phage type was observed. In 26 cases without hospital infection a strain could be isolated twice and in 14 of these a change of type was registered.

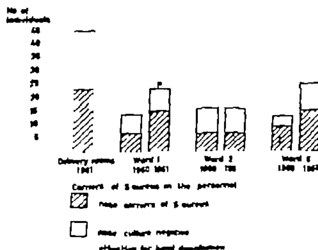


Fig _ Carriers of S. aureus in the personnel of the maternity wards of Karolinska Hospital.

TABLE 2. *Strains of S aureus isolated from individuals with probable possible and no hospital infection grouped according to phage pattern*

	S. aureus phage pattern					
	Group I		Group II	Group III		
Individuals with	Total	With 80/81 in pattern		Total	With 47 in phage pattern	Non typeable
Probable hospital infection	15	8	6	14	8	19
Possible hospital infection	2	1	2	3	2	8
No hospital infection	38	9	22	43	19	47

TABLE 3 *Comparison of strains from different site phage type similar / phage type dissimilar*

	Mother nose	Mother nipples	Child nose
Mother nipples	13/12		
Child' nose	16/18	22/14	
Child' groin	11/8	11/9	18/14

Nose carriers among the staff Among 115 members of the staff 48 (4_%) were nose carriers on some occasion (Fig 2) In 35 members of the staff S aureus was isolated twice In 13 of these cases a change of phage type was observed. One childrens nurse in Ward no I who during the previous investigation was a nose carrier and probably a spreader of S ureus and who had prior to this later investigation been treated once daily for two fortnightly periods with a nasal cream (Nebacetin, Lundbeck) containing neomycin and bacitracin, still was a carrier of S aureus in this investigation, with a strain of the same phagetype and antibiogram as in previous investigations. Of the other 11 childrens nurses—working full time in the nursery—5 were carriers on at least two occasions. The strains from the staff seemed similar as to phage groups and antiobiograms to those of the patients.

Phage types and antibiograms of all strains isolated in this investigation are compared in Table 5

TABLE 4. *Incidence of hospital i fections in mother and child related to mother s carrier state at adm ttance*

Clinical condition in mother and child	Mother carrier of S aureus t admittance	Mother not carrier of S. aureus at admittance
Probable hospital infection	3	9
Possible hospital infection	2	7
N hospital infection	33	76

TABLE 5 *Comparison between patterns of resistance and phage. (Strains from patients and personnel)*

Phage group	Patterns of resistance: Sensitive in all tests	Resistant t penicillin	Resistant t or more antibiotics	Others
I	40(13)[a]	14(4)[a]	12(6)[a]	1(1)[a]
II	23	8	—	—
III	53(24)[b]	19(6)[b]	1(1)[b]	3
Non typeable	60	2.	3	1
Sum	176	[illegible]0	16	5

Number within brackets:

[a] with phage type 80/81 in pattern

[b] with phage type 47 in pattern

Discussion

The hands of the personnel is considered a main source of infection in the maternity wards [8]. For hand desinfection pHisoHex has been proved more effective than soap [2, 3]. Introduction of pHisoHex for hand washing for staff and mothers did not significantly reduce the numbers of carriers or infection rate (Tabl. 1, Figs. 1 and 2). In most other studies on methods to lower infection and carrier rates other measures besides hand washing with pHisoHex (i.e. more strict hygiene, washing of children with hexaclorophene, disinfection of navels) were taken at the same time, which makes it difficult to evaluate each measure [6]. In this investigation the introduction of pHisoHex for hand washing was the only new measure taken to reduce carrier and infection rate, and this measure was taken in only one ward, while in the two other wards no new measure at all was taken. This makes the value of hand washing with pHisoHex easier to evaluate, and unfortunately this measure did not a single decrease the carrier frequency or the frequency of hospital infections. This of course can be explained in several ways. As mentioned one of the childrens nurses in the ward where pHisoHex was used was a chronic carrier of S. aureus. Forty-two per cent of the personnel were carriers. The staff could not wash their hands between handling of each infant (they stated that it would be too irritating to the skin). In the wards L. to 16 children were placed in one room. The traffic in the corridor was intense. All these factors must be considered when discussing methods to decrease infection and carrier frequency.

Have hospital strains of S. aureus really caused the cases registered as probable hospital infections in this investigation? Unfortunately from most infections occurring at home no cultures could be obtained. From what is known of the epidemiology of S. aureus it seems, however, probable that most of these cases are caused by hospital infection [1, 6]. As in

many other studies there was no significant correlation between carrier state and later infection—some unknown factor seems to influence the host-parasite balance

Mothers carrying S aureus in their noses at admittance to the hospital were not protected against hospital infections (Table 4) or colonization with hospital strains.

Breast feeding being the rule during hospital stay a resemblance between strains from mothers nipples and childrens noses was expected and found (Table 3) Differences are, however not uncommon and can be explained by direct spread from staff to mothers and through airborne strains.

The phage type patterns and antibiograms are similar to those observed at the earlier investigations in this hospital However phage type 47 did not this time seem to cause infection more often than other phage types. Even if strains of phage type 80/81 were isolated more often from infected than from healthy patients they did not cause any severe infections. Neither were they more resistant to antibiotics than other strains. The relatively high incidence of sensitive strains and the almost entire absence of completely resistant ones reflects the restricted use of antibiotics in the wards.

Is the observed incidence of infections and carriers tolerable? Lower as well a higher figures have been reported [1 6 8] The infections have mostly been mild. No home or hospital epidemic occurred The environment is however strongly colonized and should an epidemi strain appear the situation might easily turn critical.

New steps to reduce the contamination should be considered. It seems important that these are taken one at a time (if the purpose is to study the effect of one measure) and controlled in clinical and bacteriological investigations.

Successful therapy of carriers is difficult [5]. To force the staff to use a nasal cream as a routine is hardly practicable Too strict or uncomfortable rules tend to be neglected. Disinfections of the navels of the babies must be considered as well as the use of a disinfecting nasal cream or skin powder for the babies. We have hitherto hesitated to bath the children in some sort of disinfectant, as we have good experiences with the "conservative skin care recommended by the American Academy of Pediatrics. We refrain from using antibiotics in prophylaxis, as this would probably induce resistant strains. The new penicillinase stable penicillins could be considered less dangerous from this point of view [7] However if these penicillins are effective against S aureus they could lead to a situation, where a gram negative flora of true penicillin resistant bacterias took over the role as hospital infectants. The risk of sensitizing patients and personnel should also be kept in mind

Summary

pHisoHex for hand washing did not materially reduce hospital infection or carrier states with S aureus in the maternity ward of Karolinska Hospital. Hospital infections probably caused by S aureus were registered in about 13 % of mothers and infants during the month after delivery They were mostly mild. Five to six days after delivery 70 % of the infants were nose carriers of S aureus, mothers were carriers in 22 % of cases on admission

TABLE 5 *Comparison between patterns of resistance and phage (Strains from patients and personnel)*

Phage group	Patterns of resistance: Sensitive in all tests	Resistant to penicillin	Resistant to 2 or more antibiotics	Others
I	40(12)[a]	14(5)[a]	12(6)[a]	1(1)[a]
II	25	5	—	—
III	53(14)[b]	19(6)[b]	1(1)[b]	2
Non typeable	60	2	3	1
Sum	178	0	16	5

Number within brackets
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[b] with phage type 47 i pattern

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Summary

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and in 67 % of cases on discharge Forty two per cent of the personnel were carriers many probably chronic Strains of phage type 80/81 were more common in individuals who later developed infections than in healthy persons. Most strains were sensitive to ordinary antibiotics. Measures to prevent infections and colonisation with S aureus are discussed.

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Arterial Blood Sampling in Small Infants

by AGNETE THOMSEN *Reg Techn.*

Introduction

The conventional methods for obtaining blood samples from very small infants are often difficult and sometimes risky One practical difficulty concerns the method by which negative pressure is applied to the collecting system. Use of a glass syringe for this purpose requires a second person to pply and maintain the proper amount of pressure in order that blood will flow readily and the vessel will not collapse Use of a propipette as advocated by Lopez Banus [2], while providing a means of controlling negative pressure exerted in this system has the disadvantage of requiring a cumbersome array of equipment (tubing, needles, tubes and forceps) and still requires a second person to assist

A second difficulty with existing methods is the choice of site for sampling Femoral arterial or venous puncture carries a definite risk of deep infection [1] or thrombosis. Capillary blood obtained by heel stick also carries a significant risk of infection. In addition, in infants with poor circulation so-called arterialized capillary blood may not be truly representative of arterial blood.

The use of the temporal artery [3] as a sit of blood collection has the advantages of providing arterial blood and of reducing the risk of serious complications. In this report we wish to describe the details of a procedure which has been developed in the Premature Nursery of the Babies Hospital by which arterial blood has been obtained from this site by a single unassisted operator

The method involves use of a scalp vein set with negative pressure being applied to the system by means of a gas-tight syringe This technique has the advantage that a single operator can apply any given degree of negative pressure to the system by withdrawal of the plunger and this pressure will be maintained by the frictional forces between barrel and plunger of the syringe

Procedure

Table I shows the equipment required for temporal arterial puncture and the suggested sources of supply Proper illumination is essential and for this purpose we have found the Tensor lamp to be

This work was supported by the Health Research Council of the City f New York, under contract U 1118.

TABLE 1 *Materials needed for temporal arterial puncture.*

Materials	Vendor
Winged scalp vein infusion set No. 21 or 23 gauge needle	Abbott Laboratories, North Chicago Illinois
Hamilton gas-tight syringe* capacity 1 ml	The Hamilton Company Inc. Whittier California
Tensor lamp, Model 5973	Tensor Electric Company Inc. 1873 Eastern Parkway Brooklyn 33, New York
Heparin sodium solution 1000 U.S.P. Units	Connaught Med. Research Lab. Toronto Canada
Virac	Ruson Laboratories, Inc (Oregon) Portland 2, Oregon
Buckshot No. 5 (Flattened with a hammer)	

*The teflon tip of the syringe plunger shown should be cut off in order to reduce the dead space

quite satisfactory The steps of the procedure are as follows (Fig 1)

1 Prepare the syringe by removing the plunger and inserting the flattened buckshot in the barrel, replacing the plunger and filling the dead space of the syringe with the heparin solution.

2. Attach the scalp vein set to the syringe.

3 Shave the temporal area and clean it thoroughly with Virac solution (1:2 dilution) followed by 70% ethylalcohol. The area should be left moist since the pulsations of the artery are more easily visualized under these conditions.

4. Insert the needle into the artery preferably against the direction of flow of blood, although this is not essential.

5 With the Hamilton syringe create slight negative pressure in the system (equivalent to withdrawing the plunger to about the 0.25 ml mark)

6. A successful entry of the artery will be revealed by the flow of blood into the plastic tubing. As the blood reaches the end of the tubing, the syringe is rapidly disconnected, the air in it discharged, and the syringe reconnected and filled with blood. During filling a slight withdrawal of the plunger is usually necessary to ensure continuous flow

7 Remove the needle from the vessel and apply sustained pressure over the area for several minutes until hemostasis is complete.

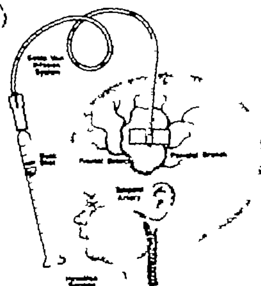


Fig. 1 Puncture of a temporal artery utilizing the equipment described in the text.

8. Disconnect the syringe from the tubing, cap it and invert it several times to insure complete mixing. Samples for determination of various acid-base parameters may be taken directly from the syringe into the Radiometer AME 1 instrument.

The above procedure has been in continuous use in the Premature Nursery of the Babies Hospital for the past year. The author has performed nearly 1 000 arterial punctures with an overall successful average of 70–80%. Most of the unsuccessful attempts occurred in newly born infants in whom facial edema obscured the vessel. However this usually diminished to the point where a successful puncture could be carried out after a period of 1 to 24 hours after birth. Repeated punctures of the artery (up to 35 times) have been carried out on some infants and infants with weights as low as 500 g have been successfully sampled.

The importance of arterial versus so-called "arterialized" capillary blood analysis with respect to acid base parameters

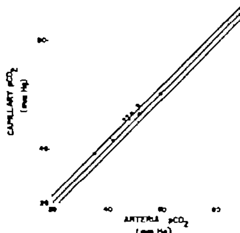


Fig. 3. Comparison of simultaneous arterial and capillary values for plasma pCO_2. The shaded area represents the estimated error of the method used.

is illustrated by data shown in Fig. 2 and 3. The data represent comparisons of blood pH and plasma pCO obtained on simultaneous arterial and arterialized capillary blood samples from an unselected group of premature infants. In both figures an appreciable number of points show a deviation from the line of identity and these deviations are outside the range of error of the method (indicated by the shaded region). Hence 10 of 16 pH values showed a significant but variable lowering of capillary pH compared to arterial values while 9 of the 14 pCO values showed significantly higher capillary values than were obtained in arterial samples. In most instances, these discrepant results were obtained from infants in whom the circulation to the extremity was judged to be poor by clinical criteria. It is of course in this group of infants that acid-base studies are likely to be of value and such findings therefore emphasize the need for reliable arterial samples.

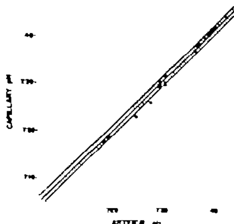


Fig. 2. Comparison of simultaneous arterial and capillary values for whole blood pH. The shaded area represents the estimated error of the method used.

Summary

An easy safe method is described for the sampling of blood from the temporal artery of small infants. Acid base studies of temporal arterial blood have revealed a number of instances where arterial blood gave significantly lower values for pCO_2 and higher values for blood pH than did simultaneous samples of capillary blood.

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Some Parameters of Respiratory Metabolism in the First 3 Days After Birth

by K ZNAMENÁČEK and H. PRIBYLOVÁ

Profound changes in adaptive processes occur in the newborn infant in the first 3 days of extra uterine life particularly with regard to respiratory metabolism. We have followed the developments in oxygen consumption, respiratory rate and rhythm and body temperature during this period, as well as the course of blood glucose lactate and pyruvate levels, the ratio of the two latter and the calculated excess lactate in capillary blood This report concerns findings in healthy new born infants up to 3 days of age following normal pregnancies and births.

Methods

Biochemical tests were carried out on blood from the umbilical vein (time 0) and subsequently on capillary blood taken at 3, 6, 12, 24, 48 and 72 hours of age. The infants were given small amounts of water during the first 24 hours and then put to the breast. Oxygen consumption was measured in a closed-system met bolimeter (Fig. 1), by a method modifying that described by Karlberg [5]. Since oxygen consumption reacts rapidly and sensitively to bodily activity and crying consumption was calculated only from periods f quiet during the hour-long determinations in the meta bolimeter Environmental temperature within the pparatus was maintained at 24 –25 C The infant was dressed lightly with pneumographic cuffs lightly fixed about the thorax to record thoracic and bdominal breathing.

Capillary blood samples (heel prick) were used for ultra-micro determinations f glucose [6], lactat and pyruvat [3]. Ultramicro modifications of these methods were worked out by Novák [7]. Excess lactat was calculated using the formula f Huckabee [4]: $XL = (L_n - L_0) - (P - P_0)$. L_0/P_0, L_0 and P in this equation were taken as verage values in healthy newborns with uncomplicated deliveries and following nor mal pregnancies, as determined at 7 hours f age In a large series $L = 19.04$ mg%, and $P = 1.95$ mg%, appeared to be valid normal values for L_0 and P_0, resulting in an L/P ratio f 9.97

Results

Table 1 summarizes findings in infants from birth to 7_ hours of age: oxygen consumption (VO_2) respiratory frequency (f) body temperature (t) blood glucose (G) pyruvate (P) lactate (L), the L/P ratio and excess lactate (XL).

Oxygen consumption fell from birth to 3 hours, some 10.5 ml/kg/hr and then rose gradually but significantly to reach its highest level t _4 hours of age (368.5 ml/kg/hr) The respiratory rate fell during

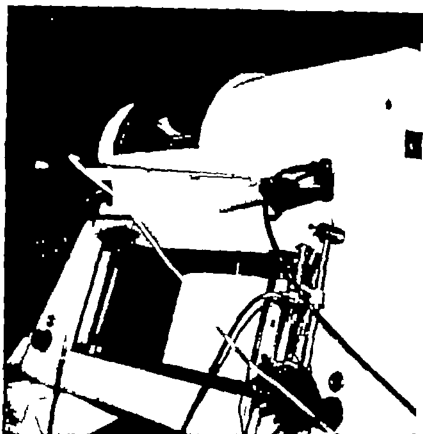


Fig 1 Closed-system metabolimeter (Czechoslovak manufacture) for determination of oxygen consumption. This apparatus simultaneously records the respiratory movements, cry and bodily activity and controls the air temperature thermostatically

the first 6 hours after birth from 54 to 46 per minute on the average and did not change significantly later during the period of observation. Rectal temperature fell about 0.5 during the usual nursing procedures in healthy full term newborns up to 3 hours of age returned to original levels by 1_ hours, and thereafter remained at about 36.5 The blood-glucose level at birth decreased abruptly to an average of 55 mg% during the first 12 hours, and then gradually rose to a normal neonatal level of about 70 mg%. Blood lactate exceeded normal values only within the first 3 hours after birth and subsequently fell slightly over 3 days On the other hand, blood pyruvate showed a sharper fall during the first 6 hours and its level tended slowly to return to normal newborn levels—2 mg —by the end of the third day During the first 3 hours the L/P ratio increased slightly and a tendency to fluctuation in equilibrium between blood lactate and pyruvate was apparent. Excess lactate decreased continuously during the first 3 days of life and virtually disappeared by the third day (1 3 mg* calculated *XL*)

Several of the relationships found are illustrated graphically In Fig the

TABLE 1 *Oxygen consumption* (VO_2) *respiratory rate* (*f*) *body temperature* (*t*) *blood glucose* (*G*) *lactate* (*L*) *and pyruvate* (*P*) *ratio of the latter* (*L/P*) *and calculated excess blood lactate* (*XL*) *in normal newborn infants without abnormalities of pregnancy or labor measured from birth* (time 0) *to 3 days after birth*

Age in hours		0	3	6	12	24	48	72
VO (ml/kg/h)	N	44	44	44	44	44	31	23
	M	325.45	314.93	324.77	333.30	368.48	365.94	34[illegible].61
	SE	6.72	9.96	10.83	10.67	10.18	9.94	11.08
f (/min)	N	33	4[illegible]	43	41	43	30	1
	M	54.87	48.60	48.28	47.93	49.14	47.48	47.04
	SE	2.09	[illegible]	1.09	1.90	1.54	[illegible]0	2.23
T (°C)	N	43	43	43	43	42	34	23
	M	36.0[illegible]	35.63	35.60	36.14	36.40	36.44	36.49
	SE	0.11	0.08	0.09	0.09	0.1[illegible]	0.10	0.10
G mg %	N	4	7[illegible]	65	64	65	35	51
	M	65.90	57.24	54.34	55.31	58.78	63.62	69.14
	SE	2.41	2.19	2.08	1.89	1.96	[illegible].61	2.87
L mg %	N	74	72	65	64	64	35	50
	M	32.52	23.41	21.18	21.94	21.75	20.05	19.04
	SE	1.10	0.65	0.60	0.[illegible]0	0.75	1.20	0.80
P mg	N	74	73	63	64	65	34	50
	M	2.52	1.96	1.81	1.86	1.81	1.90	1.95
	SE	0.09	0.06	0.07	0.08	0.07	0.11	0.08
L/P	N	70	68	63	63	61	33	50
	M	13.48	14.50	13.55	13.31	12.06	10.93	9.97
	SE	0.29	0.39	0.47	0.4	0.40	0.44	0.33
XL mg	N	73		63	64	63	33	49
	M	9.18	7.04	5.99	4.85	4.49	[illegible]70	1.78
	SE	0.80	0.55	0.60	0.54	0.48	0.54	0.79

analogous course of falls in respiratory rate and body temperature during the first day of life are shown. It is interesting that the highest incidence of periodic breathing (usually periodic hyperapnoea) was found during the first 24 hours after birth. During the period of most rapid change the blood levels of lactate and pyruvate were determined at frequent intervals (5 15 30 60 and 120 minutes after birth) in order to examine their relationship more closely (Fig 3). In order to facilitate comparison of curves, all values are expressed in millimols, the initial levels being those found in maternal venous blood drawn at the end of the second stage (crowning of the head). Stable equilibrium between lactate and pyruvat appeared between 48 and 72 hours (in the *L/P* ratio) Disturbances in this ratio were found 15 minutes after birth and disappeared between 3 and 1 hours.

Fig 4 illustrates the course of blood glucose levels and calculated excess lactate The latter reached maximal levels 15 minutes after birth simultaneously with the end of the abrupt postnatal fall in blood glucose and subsequently the

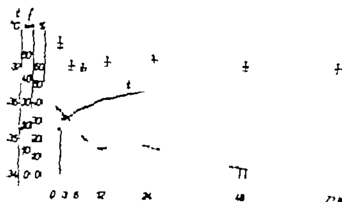


Fig. 2. The course of body temperature (t), incidence of periodic breathing (r) and respiratory rate (columns) during the first 3 days after birth.

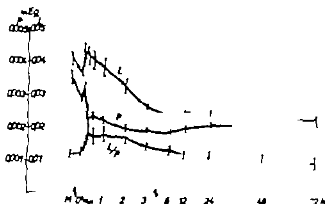


Fig. 3. Lactate (L) and pyruvate (P) in capillary blood of normal newborn infant the first 3 day of life. The lower curve shows the ratio of these (L/P)

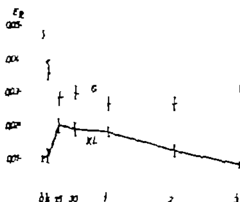


Fig. 4. Blood glucose (G) and excess lactate (XL) in normal infants during the first 3 days of life.

two curves generally pursued a mirror image course.

There was a transient decrease in oxygen consumption during the first 3 hours after birth and in the following hours a slight increase. (Table 1) The highest values for oxygen consumption in normal newborn infants were found at 24 hours of age (363.5 ml/kg/hr) at a time when XL average levels were 4.5 mg%. The highest values of XL (0.2 mg%) were determined immediately after birth when oxygen consumption was some 43 ml/kg/hr lower

(325 ml/kg/hr) The relationship between oxygen consumption and excess lactate in the blood will be examined more closely in a further report for it is in this relationship that one would expect to find reflected any significant degree of anaerobic metabolism or even oxygen debt in the newly born infant

Discussion

At definite time intervals following birth one can recognize metabolic deviations, presumably concurrent with the course of postnatal adaptation to extra-uterine life Thus, by 15 minutes after birth a sharp fall in blood glucose and pyruvate was found, together with an increase in the ratio of blood lactate and pyruvate and a rise in the calculated excess lactat in the blood. One may assume that such changes reflect a predominance of anaerobic metabolism at this period. Metabolic balance is not stabilized even by 3 hours after birth when a transient fall in oxygen consumption is found, hypoglycemia persists, body temperature reaches its low point and the L/P ratio and calculated excess lactate are still not at normal levels. During the same period, respiratory rate is lowest and periodic breathing is observed with maximum frequency Under normal conditions, it is only at the end of the third day that the L/P ratio reaches equilibrium levels, blood glucose rises to normal values, e cess lactate virtually disappears and other physiological and biochemical criteria pproach stable normal newborn values. The relative fall in blood pyruvat during a period of presumably predominant glycolytic processes immediately after birth and its relative rise after 6 hours of life seem to indicate improvement in oxidative processes This conclusion is confirmed by the concurrent fall in the L/P ratio and increase in oxygen consumption. The time relationship with frequent appearance of periodic breathing is also clear and interesting

The various relationships reported here are undoubtedly associated with a variety of neonatal adaptations—metabolic hemodynamic and so on. Reliable criteria for evaluation of the effectiveness of postnatal adaptation to extra-uterine respiration, both qualitatively and quantitatively are as yet lacking Our findings indicate that the equilibrium between lactate and pyruvate appearing in the blood, theoretically directly dependent upon tissue oxygenation, may be such a criterion. Friedmann *et al* [2] considered the L/P ratio as a measure of the relation of overall physical stress in usual daily activity Huckabee [4] has summarized the available data on average L/P ratios. 13 4 [Baumberger 1939], 13.3 [Friedmann 1945], 9.3 [Bueding 1942], 10 6 [Bay 1944]. Štembera & Hodr [8] found that the L/P ratio during normal labor varied between 5.3 and 6.6 while during prolonged labor (over 20 hours) it rose to 12. In our data, in normal newborn infants following a normal pregnancy and delivery the L/P ratio rose and stabilized t 9 97 only at * hours.

An equally important criterion of tissue oxygenation in the newborn infant should be the excess of blood lactate over pyruvate as calculated by Huckabee His formulation takes account of the entire overall summation of influences on the lactate-pyruvate system. Štembera & Hodr [8] who investigated the effect of labor

upon blood levels of glucose lactate and pyruvate found that during normal labors calculated excess lactate did not exceed 4 mg% but during protracted labor the XL might rise to 35 mg%.

In newborn infants calculating excess lactate by Huckabee s formula our results *indicate that every normal birth associated* with uterine activity is responsible for the appearance of some excess lactate in the infant. To what extent this excess lactate is due to placental transfer or arises from the infant s own metabolic activity is now under investigation. The extent to which excess lactate is related to respiratory capability in the infant also requires further study as does the role played by renal excretion in the time course of excess-lactate disappearance particularly when it is abnormally delayed.

Summary

The relationships between the neonatal *courses of oxygen consumption*, respiratory rate and rhythm body temperature and blood levels of glucose lactate and pyruvate have been investigated during the first 3 days of life. The data indicate that the L/P ratio and the calculated excess of lactate in the blood may be suitable criteria for the overall effectiveness of postnatal respiratory adaptation.

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From Clinic I and the Clinical Physiological Laboratory of the Stockholm Hospital for Infectious Diseases

Pertussis with Pulmonary Complications—A Follow Up Study

by HANS JERNELIUS

The object of the present study has been to ascertain the incidence of bronchiectasis and of disturbances of selected parts of the lung function in the follow up of pertussis patients with pulmonary complications who have received adequate hospital treatment, including careful supervision and antibiotic therapy. Bronchiectasis is often contracted during early childhood [14]. Infections, especially pneumonia and pertussis, are considered an important causative factor [14 17 23]. But the majority of studies on which this view is based have been retrospective and very few follow up studies have been made of pertussis cases.

Lees in 1950 made a radiographic study of 150 patients with pertussis [16]. Atelectasis of generally limited extent and brief duration was found in 65 cases. Six patients developed atelectasis which persisted more than t months. The at lectasis cases were studied by repeated bronchography which revealed reversible bronchodilatation in the affected lobes in 9 cases. Permanent bronchiectasis developed in one case.

Biering followed up 6_ children with pertussis complicated by pneumonia or bronchitis who had been treated at the Blegdam Hospital in Copenhagen in 1938 and 1939 [5]. The study was made nine years or more fter the illness. One case of bronchiectasis was discovered.

Williams & O'Reilly [4] followed up 37 children, who had had bronchopneumonia and simultaneous atelectasis, over periods f four to ten years pertussis had been the cause of the atelectasis in 11 cases. Bronchiectasis developed in 21 children.

No trace can be found in the literature of studies of the lung function in children with pertussis or in its aftercourse. The pathological picture is characterized chiefly by peribronchial inflammation and by areas of small atelectases and compensatory emphysema [ ]. From this starting point it is not improbable that persistent, radiographically invisible small atelectases and lesions of the bronchial walls accompanied by bronchodilatation and/or bronchoconstriction, may cause disturbances of the lung function. That bronchiectasis produces lesions which can be detected by spirometry has been demonstrated by Strang *et al* [22]. They examined 30 children and found that in the majority the forced expiratory volume in one second (FEV_1) was diminished. The appearance of the spirogram was generally of the type seen in bronchial obstruction.

This study has been supported by grant from the Swedish National Association Against Heart and Lung Diseases.

Material

The present study covers all children with pertussis complicated by bronchopneumonia and/ or atelectasis who were treated in the Stockholm Hospital for Infectious Diseases in the years 1951–1953 The total number was 58 20 of whom with radiographically proved atelectasis. The number of admissions of pertussis cases in the same period was 602. All patients were X rayed at least once during their time in hospital. Of the 58 children, 9 have not since been traced. The remaining 49 of whom 17 with atelecta sis, were examined seven to ten years after the acute illness. When taken ill, 5 of the children were less than one year of age The atelectasis children were repeatedly X-rayed during the acute phase The atelectasis was located in the right middle lobe in all except one child, in whom it was located in the left lower lobe. Atelectasis persisted for periods of ¼–9½ weeks, in five cases more than 7 weeks.

Of the 49 children traced, 9 had left Stock holm. Enquiries as to their health were made by correspondence. In no case were there symptoms calling for medical examination. The remaining 40 cases were all examined by the author The examination comprised health history physical examination, measurement of height and weight, and X ray of lungs. No case exhibited symptoms re quiring bronchography

Methods and Procedure

In 19 cases measurements were made of the forced vital capacity (FVC), forced ex piratory volume in one second (FEV) FEV % (FEV /FVC) and maximum brea thing capacity (MBC) In 18 of them the lung volumes were determined. In 5 cases, furthermore the intrapulmonary gas mixing was studied by the nitrogen wash-out method [9 18]. Half of the cases consisted f children with atelectasis of long duration or severe pertussis, the remainder were selected from the material at random. None of the subjects had had a recurrence f pneumonia since the pertussis.

Dynamic lung volumes, FVC, FEV and MBC, were measured with a spirometer made by Kifa, Stockholm, constructed on the principles described by Bernstein [3]. A 1 cm movement of the spirometer bell represents a volume of 500 ml. All determinations were made in the sitting position. All volumes were corrected to body temperature and atmospheric pressure at the water apour saturation pressure (BTPS) The MBC as read at three selected breathing rates—40, 60 and 80 breaths a minute—with the patient following a metronome, and also at a free rate f breathing The patients were instructed to breathe as deeply as possible for 15 seconds. Two readings were made at each rate, and the highest value was used. The readings of FVC and FEV were repeated so as to obtain at least three representative curves. The curve was used which gave the highest values of FEV . FEV was expressed as per cent of the highest value found for FVC. The normal values f FVC and FEV were predicted with height as coefficient of correlation from tables given by Strang [21]. he used the same type of spirometer The standard deviation for FVC is, according to Strang 0.28 litre for boys and 0.30 litre for girls. The normal alues of MBC were calculated from the formula y −9 40 34.84 H for boys and y = +5.–1 + 28.40 H for girls given by Bjure [6] (H height in metres) His study were made with the same type of spirometer The normal values for MBC were also calculated by the method described by Ferris *et al* [12, 13] and Engström *et al.* [11]. The former authors used spirometer of a different type the latter employed the Douglas bag technique

Measurements f the static lung volumes (BTPS) total lung capacity (TLC) vital capacity (VC) functional residual capacity (FRC) and residual volume (RV) were made with a closed system spirometer using helium as test gas [15, 19]. The helium concentration was read every minute with a Cambridge helium katharometer The olume of the apparatus with the spirometer bell in it lowest position was 2.33 litres. The readings

were taken with the patient in the sitting position. Only one recording was made of the FRC, but three of the VC the highest value was used. The RV was calculated by subtracting the expiratory reserve volume from the FRC. The normal values have been calculated by means f regression equations given by Engström, Karlberg & Kraepelien who employed the same method in their work. Height was used as correlation factor

The intrapulmonary gas mixing was examined by means of nitrogen wash-out graphs during oxygen breathing [9 18]. The nitrogen concentration was monitored with a Lilly N meter and Easterline Angus recorder The time for nitrogen wash-out t a concentration of % was measured.

Results

In no case the health history or physical examination has given suspicion of bronchiectasis. The lung X rays were normal in all subjects. In four cases symptoms of bronchial asthma appeared shortly fter the pertussis, but probably without causal relationship. Two of the cases exhibited other allergic manifestations as well. Four patients had had a recurrence of bronchopneumonia since discharge from hospital. One patient with severe pertussis and bronchopneumonia at one year of age developed epileptiform ttacks some weeks after discharge, which continued for six years. Ten children had had symptoms of bronchitis for periods of one to several months after the acute phase two of them up to three years. They had thereafter been asymptomatic, however and appeared to be fully healthy in the follow up.

Only one patient was slightly below the normal height and weight [7].

When examined by static spirometry the majority had values rather below the

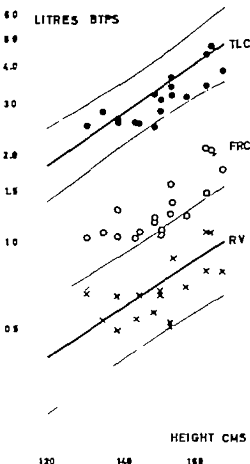


Fig. 1 Relationship between static lung volumes, litres, BTPS, and body height centimeters, in 18 children after pertussis. Regression lines with 95 per cent confidence intervals taken from Engström et al. Acta Paediat (Stockh), 45 277 1956.

predicted values for RV FRC and TLC and at least half had rather lowered VC (Table 1 and Fig. 1) With one exception the findings were above the lower normal limit ($\pm 95\%$ confidence interval) One child had a vital capacity immediately below the lower limit for the normal variation. The mean of th differences between observed and predicted values was for RV -0.09 ± 0.03 litre BTPS ($0.02 > p >$

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The intrapulmonary gas mixing was examined by means of nitrogen wash-out graphs during oxygen breathing [9 18). The nitrogen concentration was monitored with a Lilly N meter and Easterline Angus recorder The time for nitrogen wash-out to a concentration f 2% was measured

Results

In no case the health history or physical examination has given suspicion of bronchiectasis. The lung X rays were normal in all subjects. In four cases symptoms of bronchial asthma appeared shortly after the pertussis, but probably without causal relationship. Two of the cases exhibited other allergic manifestations as well. Four patients had had a recurrence of bronchopneumonia since discharge from hospital. One patient with severe pertussis and bronchopneumonia at one year of age developed epileptiform attacks some weeks after discharge which continued for six years. Ten children had had symptoms of bronchitis for periods of one to several months after the acute phase two of them up to three years. They had thereafter been asymptomatic however and appeared to be fully healthy in the follow up.

Only one patient was slightly below the normal height and weight [7].

When examined by static spirometry the majority had values rather below the predicted values for RV FRC and TLC, and at least half had rather lowered VC (Table 1 and Fig 1). With one exception the findings were above the lower normal limit (±95% confidence interval). One child had a vital capacity immediately below the lower limit for the normal variation. The mean of the differences between observed and predicted values was for RV −0.09±0.03 litre BTPS (0 02 > p >

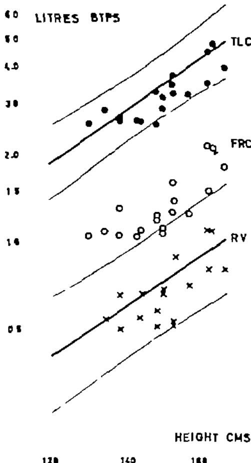


Fig 1 Relationship between static lung volumes, litres, BTPS, and body height, centimeters, in 18 children after pertussis. Regression lines with 95 per cent confidence intervals taken from Engström et al. Acta Paediat (Stockh), 46 ?77 1956.

TABLE 1 *Anthropometric data and static lung volumes litre BTPS in 18 children after pertussis*

Case No.	Age years	Sex	Height, cm	RV Observed value	RV Difference from predicted v	FRC Observed value	FRC Difference from predicted v	VC Observed value	VC Difference from predicted v	TLC Observed value	TLC Difference from predicted v
1	9	F	144	0.66	−0.03	1 16	−0.31	1 93	−0.49	64	0.19
2	10	F	150	0.65	−0.12	1 17	−0.37	2.18	−0.39	2.12	0.49
3	11	M	148	0.57	−0 17	1.36	−0.12	2.72	+0.06	3.29	−0.19
4	10	F	130	0.67	+0 14	1 06	+0.04	1.59	+0.03	2.44	+0 12
5	13	M	152	0 52	−0.27	1.63	+0.03	3.17	+0.30	3.69	0 03
6	13	M	162	0.61	−0.28	1.26	−0.34	_.94	+0.07	3.45	0.39
7	15	M	163	0 72	−0.18	1 47	−0.48	2.70	−0.78	3.48	−1 01
8	12	F	157	0.70	−0.17	1 44	−0.31	2.44	−0.70	3.14	−0 94
9	8	M	128	0 65	+0.05	1.30	+0.09	2.09	−0 11	2.74	0.13
10	9	F	148	0.61	−0.23	1 19	−0.29	2.01	−0.66	2.52	0 94
11	10	F	158	0.68	−0.09	1 07	−0.47	2.19	−0.60	2.87	0 73
12	15	F	169	0 79	−0.26	1.83	−0.35	3.09	0 75	3.88	1 11
13	13	F	165	1.09	+0.10	2.10	+0.08	3.63	+0.03	4 71	+0 03
14	8	F	128	0.50	−0.12	1.06	−0.15	2.23	+0 03	_.73	0.14
15	10	M	153	0.68	+0.07	1.36	−0.27	2.32	−0 60	3.20	0 37
17	10	M	143	0.55	−0.13	1.05	−0.29	2.17	−0.26	_.72	−0 43
18	10	M	134	0.54	−0.03	1.10	−0.02	2.26	+0.25	2.80	0 18
19	16	F	163	1 09	+0.13	2.12	+0.31	3.40	−0 06	4.49	0.03
Mean difference				−0.09±0.03		−0 18±0 05		−0.26±0.08		−0.41±0.10	
p				$0.03 > p > 0.01$		$0\ 01 > p > 0.001$		$0\ 01 > p > 0\ 001$		$p < 0\ 001$	

0 01) for FRC $-0\ 18 \pm 0\ 05$ ($0\ 01 > p > 0\ 001$) for VC $-0.26 \pm 0\ 09$ ($0\ 01 > p > 0\ 001$) and for TLC $-0\ 41 \pm 0\ 10$ ($p < 0\ 001$) litre BTPS The standard deviation for normal standards is 19 7 % for RV 15 4 % for FRC 12 6 % for VC, and 12.2 % for TLC, if the height is used as correlation factor [10]

In dynamic spirometry (Table 2) the FVC of 8 children was below the normal limit (-2σ) The mean of the differences between observed and predicted values was $-0\ 41 \pm 0\ 10$ litre BTPS ($p < 0\ 001$) The mean $FEV_{1.0}$ ratio ($FEV_{1.0}/FVC$) for the entire material was 86 %. Strang gives the normal value for boys as 85 ± 6 % and for girls 80 ± 5 % [21] Only one patient showed a definitely lowered $FEV_{1.0}$ ratio of 71 %

In 13 out of 19 subjects the maximum breathing capacity (MBC) proved to be below the lower limit for the normal variation reported by Bjure [6]. The mean difference between observations and predicted values was $-4? \pm 5$ litres BTPS/min which is significant ($p < 0\ 001$). If the observations of MBC are instead compared with predicted values according to Ferris *et al* [1? 13] or Engstrom *et al* [11], low values are found only in t o cases. These two children, however achieved a normal MBC at a rate of 80 breaths per minute in time with a metronome The mean difference between observations and

Table 2. *Results from dynamic spirometry in 19 children after pertussis*

Case No.	FVC, l BTPS Observed value	FVC, l BTPS Difference from predicted v	FEV ₁/FVC % Observed value	FEV ₁/FVC % Difference from predicted v	MBC, l BTPS Observed value	MBC, l BTPS Difference from predicted v F	MBC, l BTPS Difference from predicted v B
1	1.95	−0.60	92	−6	77	+3	−2[illegible]
2	1.80	−0.89	82	4	54	−18	−36
3	2.48	−0.45	95	+12	53	−29	−60
4	1.93	+0.15	90	+4	52	−6	−15
5	2.70	0.18	86	0	4[illegible]	−20	−89
6	2.92	−0.28	90	−4	85	−1	−39
7	3.01	−0.74	89	−3	138	+11	−44
8	2.64	−0.84	71	−15	66	−29	−61
9	2.83	+0.03	84	−2	77	+9	+23
10	2.20	−0.65	78	8	63	−15	−44
11	2.26	−0.74	98	+1	67	−23	−44
12	2.92	−1.[illegible]1	68	12	102	−5	−54
13	3.50	−0.42	84	2	80	−43	−67
14	2.26	−0.09	78	8	83	−19	−35
15	2.42	−0.84	93	7	74	−12	53
16	4.39	+0.61	79	[illegible]	163	+36	−26
17	2.20	−0.80	78	8	74	−8	−35
18	2.37	+0.08	85	−1	84	+15	+1
19	3.41	−0.4[illegible]	88	2	87	−16	−54
Mean diff		−0.41 ± 0.10		0.4 ± 1.7		−8 ± 5	−4[illegible] ± 5
P		< 0.001		> 0.05		> 0.05	< 0.001

F predicted value from Ferris *et al* [12, 13].
B predicted value from Bjure [6].

predicted values according to Ferris *et al* was −8±5 litres BTPS/min. (non-significant) and according to Engström *et al* +5±5 litres BTPS/min. In a smaller series of [illegible]0 healthy schoolchildren examined at the hospital with a Bernstein spirometer [4] the values of MBC were very much lower than those found by Bjure but in close agreement with those of Ferris *et al*. It seems best therefore to compare the MBC of the pertussis cases with the standard values of Ferris *et al.*

The intrapulmonary gas distribution was examined in five cases (four had had atelectasis and one bronchopneumonia). All exhibited rapidly falling wash-out curve and a nitrogen concentration below in expiratory air was attained in less than [illegible] minutes, indicative of a homogenous mixture of gases in the lungs [1].

Discussion

The static lung volumes have proved to be on an average slightly lower than the predicted normal values in the group studied. The diminished total lung capacity is due to a lowering both of the residual volume and of the vital capacity. The difference between observed and predicted values is statistically significant but the absolute figures are small and lie within the normal variation. The changes are so small that one is not justified in drawing

any conclusions as to their implication. In all cases but one the individual values are within the normal variation. The one exception, a 10-year-old girl of slender build had a vital capacity immediately below the lower normal limit In the follow up she exhibited no clinical symptoms of pulmonary disease

The results of the dynamic spirometry have shown normal $FEV_{1.0}$ ratio and there is thus no increased resistance to breathing Eight children had a pathologically low FVC Seven of them, however had a normal vital capacity when examined with a helium spirometer Their low FVC are probably due to the fact that it is more difficult to get children to cooperate in this test than in tests of ordinary vital capacity One of the children was reported by her mother to become short of breath during exertion She also had a lowered $FEV_{1.0}$ ratio of 71 %. In this case the lowered values were probably due to extrapulmonary factors. Functional tests showed a low working capacity (w_{170}) [?0] probably due to muscular factors. Exercise electrocardiogram showed no abnormality The remainder of the patients were entirely free from symptoms in the follow up

The MBC values are normal if compared with our own small standard material and with predicted normal values according to Ferris *et al* [1° 13] and Engström *et al.* [11], but are mostly abnormal if compared with Bjure s [6] standards. The very much higher level of Bjure s figures is probably due to the fact that in his study the children chose or were instructed to breathe at a quick rate of between 80 and 90 breaths per minute In our material the children breathed at an average of 50 breaths per minute

To sum up it may be concluded that children who have had pertussis with pulmonary complications have on an average smaller static lung volumes than normal children. However the difference is small and might be explained by a slight systematic disparity of the materials, as the number of patients in the present study is small Signs of increased resistance to breathing are absent, and the maximum breathing capacity is within normal limits.

The study appears to show that pertussis complicated by bronchopneumonia or atelectasis, if adequately treated, does not predispose to bronchiectasis or pulmonary diseases. The result of this study is in good agreement with those of Lees [16] and Biering [5] who found that the risk for bronchiectasis after pertussis was small The reversible bronchiectasis reported by Lees [16] in cases of pertussis with atelectasis might explain the protracted symptoms of bronchitis after the acute phase in some of the present cases. The conception that pertussis is a common cause of bronchiectasis in children is based on retrospective studies and seems not to be correct. Repeated infections in bronchi and lungs to a great degree facilitate the origin of bronchiectasis. It is possible that late complications were more common in the past, when children s resistance against infections was smaller and antibiotics were not available The risk for late complications will probable increase if the treatment is neglected. Therefore it is still important that pertussis patients with pulmonary complications are adequately treated with antibiotics, and cases with atelectasis should be kept under careful observation and as much as possible protected from infections.

Summary

Forty nine children with pertussis complicated by bronchopneumonia or atelectasis were examined seven to ten years after the acute illness. The examination comprised health history physical examination and X ray of the lungs. Static and dynamic spirometry was also performed in 19 cases, and the intrapulmonary gas mixing was examined in 5 cases.

No case of bronchiectasis or other sequelae was found. The static lung volumes in the group studied were on an average lower than the predicted normal values. The difference was small but significant. With a single exception, however the individual values were within the normal variation. The result of the dynamic spirometry showed there to be no increased resistance to breathing The maximum breathing capacity and the intrapulmonary gas mixing showed normal values.

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Auto-Immune Thyroiditis in Children and Adolescents
I Clinical Studies

by LARS R. NILSSON and DEBORAH DONIACH

In a study of adolescent goitre under taken in Gothenburg in 1959 several children were found to have unusually firm goitres. Using thyroid biopsies and immunological tests for thyroid antibodies, these cases were shown to have lymphoid thyroiditis. The application of these methods was then extended to most other cases of non-toxic goitre seen at the Gothenburg Children s Hospital September 1959–February 1963 and it became evident that uto-immune thyroiditis is a common cause of thyroid enlargement in children and adolescents, in western Sweden. The results of the cytological aspiration biopsies will be reported (Nilsson & Persson, 1964) The present paper i concerned with clinical and thyroid function studies in 35 cases of juvenile lymphocyti thyroiditis and a further communication will include the detailed result of auto-antibody investigations in the patient and their parents (Doniach Nilsson & Roitt 1964)

The term " uto-immune thyroiditis" has been adopted to cover lymphocytic lesions of varying se erity and extent Classical Hashimoto s disease or struma lymphomatosa is unusual in young subjects and many have a milder variant of the disease A few patients with juvenile myxoedema having no thyroid enlargement were included since this type of thyroid atrophy has been shown in adults to be closely related histologically and immunologically to the goitrous variants of lymphoid thyroiditis.

Material and M thods

Patients The series includes 29 cases studied in Gothenburg and 6 patients similarly nvestigated in London. The total group consist of 6 boys and 29 girls aged between 7 and 17 t the time of study T ble 1 gives detail of all the patient included in the present report Tabl 2 shows the incidence of lymphocytic thyroiditis among all thyroid patient aged 0–16 seen at the Gothenburg Children Hospital during the period of this study

Methods Cytologic aspiration biopsies with fine needle were performed in Cases 1–26; Cases 29 31 33 and 34 had histological needle biopsies performed with the Turner Warwick biopsy instrument (El Kabir *et al.* [18]) and the remaining cases had no goitre t the time of study

I^{131} thyroid uptakes were performed in the

TABLE I *Clinical data* [illegible]

Init.	Sex	Age on admission	Known duration	Goitre: Size	Goitre: Consistency	Goitre: Surface	Clinical thyroid status	Metabolism: BMR (+15 to −15)	Metabolism: PBI γ (4–8μg%)	[illegible]
LP	F	½	1 year	M	firm	bossel.	eu	+2	[illegible]	[illegible]
RG	F	9	3 yrs	M	firm	bossel.	eu	+–, −5	[illegible]	[illegible]
EN	F	9	1 year	M	firm	smooth	eu	−4, −6	[illegible]	[illegible]
IF	F	10	1 mth	M	mod.	nod lobe	eu	−3	[illegible]	[illegible]
SLE	M	11	1 mth	M	firm	bossel.	eu	−7 −11	[illegible]	[illegible]
IT	F	11	1 year	M	firm	smooth	eu	+2, −8	[illegible]	[illegible]
KO	F	11	1 year	M	mod.	smooth	eu	–	[illegible]	[illegible]
KW	F	11	2 mths	S	mod.	smooth	eu	−3	[illegible]	[illegible]
LA	F	11	1 mth	L	firm	bossel.	eu	−–, +12	[illegible]	[illegible]
MA	F	12	8–9 yrs	M	firm	bossel.	hypo	+5, −4	102	[illegible]
ASH	F	1	1 year	M	mod.	smooth	eu	+10, +7	[illegible]	[illegible]
BB	F	12	1 mth	S	soft	smooth	eu	−3	[illegible]	[illegible]
YH	F	13	mths	M	mod.	smooth	eu	+10, +1	[illegible]	[illegible]
BP	F	13½	2–3 yrs	L	firm	bossel.	hypo	−10	[illegible]	[illegible]
MO	F	13½	6 mths	M	mod.	bossel.	eu	+1	[illegible]	[illegible]
IO	F	14	1 mth	L	firm	bossel.	eu	−4	[illegible]	[illegible]
KL	F	14	mths	S	mod.	smooth	eu	−6	123	[illegible]
CL	M	14	6 mths	M	soft	smooth	eu	−1	[illegible]	[illegible]
RO	F	14	3 yrs	M	mod.	bossel.	eu	−10, [illegible]	[illegible]	[illegible]
DH	F	14½	3 years	L	firm	bossel.	eu		[illegible]	[illegible]
AQ	F	15	years	M	mod	bossel.	eu	−5	[illegible]	
BME	F	15	1 year	S	soft	smooth	eu	−6	[illegible]	[illegible]
BH	F	15	3 mths	M	mod.	smooth	eu	−8	[illegible]	[illegible]
LJ	F	15	1 year	L	firm	bossel.	eu	−12, 14	[illegible]	[illegible]
BG	F	15½	1½ yrs	L	mod.	nod.	eu	−12	[illegible]	[illegible]
RB	M	16	1 year	M	soft	smooth	eu	[illegible] 6	[illegible]	[illegible]
KB	F	17	3–4 yrs	M	soft	smooth	hypo	[illegible] 10	[illegible]	[illegible]
SR	F	13			N goitre		hypo?	−19	[illegible]	[illegible]
MO	M	7	1 mth	M	mod.	smooth	eu		[illegible]	[illegible]
RO	F	10	4 yrs	S	mod.	smooth	myx		11	
PB	F	14	2 yrs	L	firm	smooth	eu	8, −10	61	[illegible]
CH	M	14	1 year		N goitre		myx			
UH	F	14	6 mths	M	mod.	smooth	eu	1	103	[illegible]
PD	F	16½	1 mth	M	firm	bossel.	eu	13, 9	100	[illegible]
AM	M	14			N goitre		myx	23	[illegible]	[illegible]

Normal values in brackets.

+ Fluorescent test for colloid and/or cytoplasm positive TRC 0–2500.

+ + Thyroid complement fixation titre 0–1/32 and TRC up to 25,000.

+ + + Thyroid complement fixation 1/64–1/512 with or without TRC

roid function tests

I^{131} uptake tests N	N_{24}	N_{48} (max. upt. 20–40 %)	PBI 48 hrs. (0–0.3 %/L)	γ-glob. g% (0.9–1.0 g%)	Thyroid antibody titres (all negative)*	Remarks
very low (exogen. influences?)				1.3	+	
30	47	47	0.63	1.4	+ + +	
45	51	47	1.03	1.6	+	
36	33	28	1.35	1.1	+ + +	Diabetes since age of 9
46	52	[illegible]	0.06	1.3	+	
50	56	59	0.26	1.6	Neg → +	Aspiration cytology doubtful thyroiditis but clinically suggestive
4	75	76	0.35	1.3	+ +	Subacut onset with painful goitre, 1 year later hypothyroid
70	54	53	1.5	1.3	+	Short stature, no improvement with T
32	42	39	0.56	1.5	+ +	Joint pains
12	16	11	1.57	1.5	+	Retarded growth and delayed puberty
47	56	54	0.29	1.7	+ +	1 year later goitre knobbly
43	55	55	0.3	1.3	+	1 year later goitre firmer
30	40	37	0.26	1.3	+ − +	
29	30	24	1.71	1.8	+ +	X-ray tracheal compression
16	35	36	0.52	1.5	+ +	
19	26	25	0.22	1.9	+ + −	
70	67	60	1.57	1.7	− +	Exacerbations and remissions of goitre
50	49	47	1.38	1.4	+	Anxiety and nervousness, increased pulsation of goitre
34	53	53	0.14	2.0	+	Constitutionally undersized with abnormal fingers and toes
25	30	27	0.32	2.0	− − +	1 year later mild hypothyroidism
y low (exogen. influences)				1.1	+ + +	
31	42	42		1.3	+ −	6/12 later became hypothyroid
30	39	40	0.16	1.4	−	6/12 later became hypothyroid
43	49	46	0.15	1.8	−	Goitre size fluctuating, later firm
34	43	44	0.13	1.9	+ +	Familial colloid goitre with superimposed thyroiditis. Perchlorate discharge neg.
31	34	33	0.4	1.4	+ +	Breathless on exertion, palpitations
50	46	38	1.9	2.2	− + +	Fluctuating goitre regressed spontaneously with onset of mild hypothyroidism.
31	41	42	1.2	1.3	−	Mentally retarded, dysplastic habitus
37	(I^{132})			normal	− −	Enuresis and muscle pains, goitre symptomless. 2 yrs. later goitre firm, patient euthyroid.
				raised	+	Profound myxoedema 2 yrs. Bone age 5.9 yrs
11	30		0.56	raised	+ + +	Over next 4 yrs. goitre progressively larger and patient mild hypothyroid
					+ + −	Florid myxoed. rapid onset following upper respiratory infection
29	64		0.2	raised	+ +	After 2 yrs. goitre larger and firmer patient still euthyroid AB titre increased
under	60 (high)		0.62	raised	− − +	Became myxoedematous 4 yrs. after painful onset
4	2	2	0.15	1.3	−	Diabetes at 6, growth retard. from age of 9–10 yrs. severe myxoedema at 13 yrs.

TABLE 2 *All cases of thyroid disease seen at the Gothenburg Children's Hospital September 1959 to February 1963*

Diagnosis	Both sexes	Girls	Boys
Lymphocytic thyroiditis	31	28	3
Thyroiditis following thyrotoxicosis	1	1	—
Thyrotoxicosis	4	3	1
Juvenile myxoedema	2	1	1
Congenital myxoedema	1	1	—
Iodide goitre	1	1	—
Inborn thyroid dyshormonogenesis	5	2	3
Cystic colloid goitre	3	3	—
Simple goitre	32	31	1
Total number	80	71	9

conventional manner using a scintillation counter with a tracer dose of 10–20 μC. The protein bound radioactivity in 48 hour plasma samples was separated on Amberlite Resin Ira 400. PBI 127 was determined with a modified Barker method (Skanse & Hedenskog [35]). BMR was calculated from the oxygen consumption in a Lundia spirometer model II using the standards given by Lewis *et al.* [27]. The serum proteins were estimated by the biuret method and separated by paper electrophoresis; strips were scanned a Chromoscan (Aronsson & Grönvall [1]). The results of immunological tests for thyroid gastric non-organ-specific AICF antibodies and for anti-nuclear and rheumatoid factors will be reported separately.

Results

Onset and symptoms

Goitre was the presenting sign in 31 cases, myxoedema in three and symptoms suggestive of hypothyroidism in one girl without thyroid enlargement. Age distribution at known onset is illustrated in Fig. 1; the youngest age was 3 years. The disease became manifest before the menarche in 59 % of the girls (Table 3).

The goitre was first noted at school health examinations or consultation for other complaints in nine patients. In one case it developed rapidly with local pain, heat intolerance, nervousness and general malaise. A further two girls had a rapid onset but the history was more vague. In the remaining cases the patients or their parents had become aware of the neck swelling accidentally, in two instances during an upper respiratory infection. A few patients complained of sweating, nervousness or tiredness as the first symptoms of the disease.

Symptoms attributed to the disease and

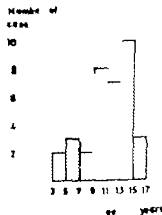


Fig. 1. Age distribution at known onset of thyroid disease (31 cases).

TABLE 3. *Thyroiditis in relation to onset of menstruation in 29 girl*

	No. of patient	Age at onset of menstruation in years: Median	Range	Remarks
Thyroiditis onset before menarche	I	1.. (10 cases)	11–12.6	Seven not yet menstruating of whom two aged 14.. and 14 6 ? delayed menarche
Thyroiditis onset t menarche	3	13 0	11 14	
after menarche	9			
Controls with adolescent colloid goitres	25	1	10.. 14.3	

their frequency are shown in Table 4 Thirteen patients had no general complaints and most of the other cases had mild disturbances which could not always be attributed to the thyroiditis. Two types of general symptoms were recognizable as indicating hypo- or hypermetabolism feeling cold, tired and dull were conspicuous in the myxoedema cases but similar milder symptoms were present in about one-fourth of patients who were not obviously hypothyroid. The other group of symptoms included sweating moderate heat intolerance nervousness and general lassitude These appeared at the onset in some cases or in connection with obvious exacerbations with increase in goitre size Slight mental changes such as

TABLE 4 *Symptoms n 35 cases of juven le uto- mmune thyroid tis*

Neck pressure or pain	9
Increased sweating	7
Cold sensitivity	11
Fatigue and dullness	1
Behaviour changes	6
Breathlessness and palpitations on moderat physical exertion	2
Mild neck symptoms only	2
N symptoms	11

irritability and restlessness are difficult to evaluate and were noted in Table 4 only when the parents stressed their recent onset or distressing nature Intestinal upsets were not reported. A few girls had mild menstrual irregularities which could not be referred with certainty to the thyroid disease

Nine patients complained of heaviness or pressure in the neck on swallowing three also having neck pain at the onset or during exacerbations and one had dysphagia with tracheal compression. However in 23 of 32 cases the goitre was symptomless Apart from true exacerbations, the size of the goitre was said to fluctuat in some cases with physical effort mental stress and menstruation, or without apparent cause

Cl nical signs

Goitre. Individual findings are shown in Table 1 and are summarized in Table 5 Small goitres were easily palpable but only visible on careful inspection. Medium-sized goitres were visible and estimated to be 2–3 times larger than the normal gland. Large goitres were quite obvious with a vertical lobe height of 5–7 cm Increased

TABLE 5. *The character of goitre in 35 patients with auto-immune thyroiditis*

	Consistency			Surface			
Size	Firm	Moderate	Soft	Nodular	Bosselated	Smooth	Total
Large	6	1	—	1	5	1	7
Medium	7	10	3	1	8	11	20
Small	—	3	2	—	—	5	5
No goitre							3
Total No.	13	14	5	2	13	17	35

consistency of the gland was classified as moderate or firm but none of these young patients had the really hard or rubbery goitres seen in adult Hashimoto patients. Surface irregularities classed as bosselated in 13 cases could be granular, uneven or lobular, and only two patients had definite nodules. Seventeen patients had smooth diffuse goitres, two of them vascular and reminiscent of Graves disease. All goitres were freely mobile. Pea-sized lymph nodes were palpable above the upper poles of the thyroid in some instances. Slight compression of the trachea was found on X-ray in only one girl with a large goitre causing dysphagia.

Somatic growth. The height of the patients was compared with standard values according to a Swedish growth chart (Karlberg & Iggbom [20]). Eight girls and two boys were clearly taller than the mean height for the corresponding age but only two girls fell above the 95 % confidence limit. Six children were shorter than the mean, four of them below the 95 % limit, of whom three had myxoedema. The fourth had small parents and some anomalies indicating a genetically determined short constitution. One other short girl had moderate hypothyroidism: she failed to have the normal growth spurt at 13 years of age but grew again after institution of thyroid hormone. However this is of doubtful significance since her mother also had a late puberty. There were no significant deviations from the normal weight except for the myxoedema cases who were all overweight. Median age at menarche is shown in Table 3. There was some suspicion of delayed puberty in two girls aged 14½ and in a boy of 14 who was myxoedematous.

Associated diseases. Two patients had diabetes mellitus diagnosed before the thyroid disease. One girl had abnormalities of fingers and toes, short broad hands and feet and was constitutionally underdeveloped. Another had doughy soft tissues, hyperextendable joints and subnormal intelligence. There were no associated rheumatic or allergic conditions except for one girl who complained of intermittent pains in the left wrist increasing with weather changes, one boy with muscle pains, and one girl who had repeated attacks of bronchitis which may have included an allergic component.

Laboratory investigations

Basal metabolic rate was measured in 31 patients and gave abnormally low results only in patients with myxoedema, two

TABLE 6. I^{131} *values in 31 cases of juvenile auto-immune thyroditis*

Maximum peak uptake %		No. of patients
	< 20	1
(Normal range)	20–40	11
	41–50	8
	> 50	10
48 hr PBI[131] %/litre		
(Normal range)	< 0.20	5
	0.20–0.40	8
	> 0.40–1.00	7
	> 1 00	9

girls with mild hypothyroidism had bor derline readings, and all other patients gave normal results.

Protein-bound iodine. Seven patients had PBI values of 8–12.3 μg/100 ml without a corresponding rise in BMR or symptoms of thyroid overactivity The myxoedema cases had very low values of 1 1 and 1.5 μg but in all other patients PBI values lay between 4 and 8 μg/100 ml.

Radioiod ne tests Individual findings are given in Table 1 and summarized in Table 6. There was a marked tendency to high uptake values Twelve patients had maxi mum thyroid uptakes of 50–76 % and the mean uptake for the group was 46 % excluding the myxoedematous cases The 48 hour PBI[131] was markedly raised in 16 of 29 cases (0.52–1.9 %/litre) and only 5 patients had values below 0.2 %.

Serum protein electrophoresis Total protein values were all within normal limit but 16 of 34 patients had raised γ-globulin values (1 7–_2 g/100 ml in 12/29 cases estimated quantitatively)

Erythrocyte sed mentati n rate and serum cholesterol The ESR (1 hour Westergren) was measured in 33 patients. It wa less than 10 mm in 23 cases, between 10 and 20 mm in 9 and 25 mm in one boy with myxoedema and diabetes The serum cholesterol was raised above 300 mg/100 ml only in the three children with advanced myxoedema

Clinical course and effects of treatment

Twenty four cases have been observed for over a year and some for _–4 years before institution of replacement therapy The observations on change in goitre size and thyroid status are summarized in Table 7 In seven patients the goitre slowly increased in size firmness and irregularity of the surface including four cases who had small soft thyroid swellings diagnosed as typical adolescent goitres when first seen. In nine patients the goitre showed marked variations in size and in one girl the swelling disappeared spontaneously while she became hypothyroid. A few patients complained of alternate periods of feeling cold and sweating Apart from the three cases with myxoedema and four who presented with mild thyroid defi ciency hypothyroidism developed in six patients during the follow-up In three cases this occurred within 1–2 years of the known onset of disease while in the other three patients thyroid function declined more slowly over several years.

Treatment with thyroid hormone ha been instituted in _5 of the patients. The initial dosage was 0 1 mg of L-thyroxin later increased to 0.2 mg daily in some cases. The duration of treatment has been rather short in most cases exceeding one year in only seven. The myxoedematous patients improved to full health on therapy Of nine patients considered mildly hypothyroid, seven girls felt much better

TABLE 7 *Course and prognosis in 35 cases of juvenile thyroiditis.*

	Number of patients
1 *Goitre (before treatment)*	
Regressed spontaneously	1
Increase in firmness and bosselation	5
Progressive increase in size and firmness	7
No change in size and consistency after 1 3 years	9
Follow-up less than 9 months	10
No thyroid enlargement at any time	3
Total	35
2 *Thyroid status*	
Hypothyroid t diagnosis	7
Later became hypothyroid	6
Remained euthyroid without therapy	10
Euthyroid when treatment started	1
T tal	35

on thyroid hormone a change also noticed by their parents One girl grew well during twelve months of therapy The mentally retarded girl experienced no clear improvement on treatment. With respect to itre size information is available in 15 cases An obvious decrease in size was found in nine cases of which four had been treated for only 3–4 months. In five patients no clear regression of the goitre could be registered but four of these had been on therapy for only three months. The remaining patient had an unexpected and marked doughy swelling of her goitre when seen two months after starting treat ment but this later regressed and her goitre became rather small

Incidence of thyroiditis in children and adolescents

In a goitre survey carried out on school children aged 10–16 examination of 519 boys and 595 girls revealed *definite goitres* in two girls and doubtful thyroid enlargements in four while all the boys had nor mal glands. As seen in Table of a total of 72 cases of non toxic goitre seen at the Children s Hospital in 3½ years, no fewer than 31 suffered from thyroiditis. This high proportion of 40 % was found in a selected group examined in hospital and it may be presumed that this includes the more prominent goitres or those giving rise to unpleasant symptoms and that the proportion of thyroiditis cases among all thy roid enlargements is probably lower The number of children examined was too small to draw conclusions about the incidence of goitre in the entire school population of Gothenburg which was estimated to be of the order of 32,000 for this age group.

Discussion

Hashimoto s disease has long been considered rare in childhood [6 29 31]. However Gribetz Talbot & Crawford [18] reported six cases of lymphocytic thyro-

ditis in girls and stated that this disease was responsible for about one-third of their cases of non toxic goitre among preadolescent and adolescent girls Recently this series was extended and followed up (Saxena & Crawford [33]) Loboeuf *et al.* [21] found thyroid antibodies in 7 of 31 children and adolescents with simple goitre in Philadelphia. Clayton & Johnson [7] reported the occurrence of struma lymphomatosa in 12 euthyroid children aged 7–16 years. Berglund *et al* [?] studied five cases of lymphadenoid goitre in children of whom three had definite signs of hypothyroidism In addition to these studies, cases of Hashimoto s disease in children have been reported singly or in small groups [4 10 4, 32] or mentioned in connection with th disease in adults [23 25 36 38]

The recent papers quoted above and our own investigations point to a higher incidence of auto-immune thyroiditis among children with non toxi goitre than was hitherto realized and it is remarkable that this was of the order of 40% in Boston and Gothenburg in spite of possible geographical variations. The striking increase in the reported incidence of this disease in all parts of the world since the advent of radioiodine and immunological tests is more likely to be the result of an increased awareness rather than reflect true increase in the frequency of this condition. It is a common experience that 'rare diseases become less unusual when especially looked for

Clinical aspects

The clinical manifestations of auto-immune thyroiditis in childhood and adolescence may be considered in three main groups representing different degrees of intensity in the auto-immune process and different stages in the evolution of the disease (1) subacute onset of goitre with pseudothyrotoxic symptoms, ( ) hypothyroidism with or without goitre (3) non toxic goitre without symptoms or with mild general complaints. There are transitional forms between these The first type is seldom seen and may perhaps be regarded as an unusual variant of the disease Juvenile myxoedema and classical Hashimoto goitre with hypothyroidism are also uncommon. In these first two groups the auto-immune process appears to be particularly intense, leading to destruction of the thyroid gland in a relatively short time Symptomless non toxic goitre appearing insidiously is the commonest expression of chronic thyroiditis in children. However when these patients are followed up over a period of years, a proportion become hypothyroid with or without compensatory increase in the size of the goitre An 8–10 year follow up of the Boston series by Saxena & Crawford [33] showed that 17 of their 32 cases had become hypothyroid. In the present study 13 of 35 patients are mildly or severely hypothyroid before reaching adult age and in many there has been an increase in the firmness and boselation of the goitre pointing to a progression of the disease It is therefore likely that most patients will develop hypothyroidism sooner or later although the process may become quiescent for many years in some instances [12]

The symptoms of mild hypothyroidism were often vague and the only complaint directly pointing to hypometabolism was feeling cold. Other symptoms such as

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Follow-up less than 9 months	10
No thyroid enlargement at any time	3
Total	35
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Hypothyroid at diagnosis	7
Later became hypothyroid	6
Remained euthyroid without therapy	10
Euthyroid when treatment started	12
Total	35

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Discussion

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ed tissue and release of iodinated peptides from the colloid due to acinar injury by sensitized immunocytes or cytotoxic antibodies or both. There is no doubt that in the advanced form of Hashimoto's disease seen in adults, reduction of the iodine pool is the more important and indeed it represents the transitional stage of low thyroid reserve which precedes overt hypothyroidism. However in young patients, especially those with high uptake values, histological examination of the thyroid shows fairly good colloid stores and in the early stages of the disease it is probable that acini continually break up and regenerate with leakage of iodinated proteins into the circulation. This process is mirrored in the disproportion between the serum protein-bound iodine (PBI) and its butanol extractable fraction (BEI) which is a notable feature in juvenile thyroiditis [7, 18, 33]. The estimation of BEI presents technical difficulties which prevent the use of this valuable test in many clinical laboratories. In auto-immune thyroiditis a normal PBI does not exclude the existence of mild hypothyroidism nor can a raised PBI level be used as sole evidence of thyroid hyperactivity. Owing to the appreciable amount of butanol insoluble iodine circulating, the PBI levels in children with lymphoid goitres tend to be on the high side of normal and in our series no fewer than six patients had plasma iodine values in the thyrotoxic range (0.4–1.3 μ g/100 ml). It is noteworthy that four of these six girls also had extremely high PBI^{131} levels (1.35–1.67 % per litre). Usually these children are euthyroid or hypothyroid and the question of thyrotoxicosis does not arise. However, it is important to rule out a "Graves constitution" by carrying out a triiodothyronine suppression test [41] in patients with a raised I^{131} uptake, especially in view of the frequent familial association of thyroiditis with thyrotoxicosis.

The γ-globulins do not parallel the auto-antibody levels closely and there are patients with raised globulins who have very low titres of detectable antibodies (cf. Case 19). The ESR and serum cholesterol appear to be much less commonly raised in young subjects than in comparable adults with Hashimoto goitres and were only abnormal in children with profound myxoedema.

Cases of auto-immune thyroiditis with subacute onset may be difficult to distinguish clinically from de Quervain's disease but the low or absent iodine uptake and negative thyroid antibody tests in the latter disease separate it from auto-immune thyroiditis. Mild chronic thyroiditis may be confused with colloid goitre, iodine deficiency goitre and euthyroid dyshormonogenetic goitre; antibody tests and radioiodine results allow a firm distinction to be made in most cases.

The histological criteria of chronic thyroiditis have been discussed by many authors [19, 23, 37, 4.] and special emphasis has been placed on the use of needle biopsies [8, 9, 39]. The diagnostic reliability of cytologic aspiration biopsies has been studied by Nilsson & Persson [28].

The auto-immune aspects will be discussed in the second part of this study but it may be mentioned here that thyroid antibody titres were far lower than in adult patients (see Doniach & Roitt [13]). Thyroglobulin precipitin tests were nega

tive in all cases and the highest tanned red cell titre was 1/25 000—CFT titres were moderate or high in less than one-third of the patients and in nine cases only the fluorescent tests were positive

Treatment

The treatment of choice for auto-immune thyroiditis at any age is thyroid hormone preferably L-thyroxine in full physiological dosage of 0 1–0.3 mg daily. The indication for replacement therapy is absolute in cases with hypothyroidism or those with large goitres causing pressure symptoms though opinions may differ about the institution of thyroxine treatment in every euthyroid patient with thyroiditis since it is not yet known how often the lymphadenoid process remains of low grade or goes into a quiescent phase after the first attack. In our clinics treatment was started chiefly in cases with large goitres or when the thyroid swelling became progressively firmer and irregular but also in patients with small goitres and reduced thyroid reserve as evidenced by their raised 48 hour PBI[131]. In view of the auto-immune character of this disease adrenal steroids have been tried in some cases and have produced a reduction in goitre size [2 3 20]. However the disadvantages of prolonged therapy with these potent agents for a condition which is in no way life threatening exclude this form of treatment except under special circumstances for a short time. Thyroidectomy is contra indicated in children and adolescents with thyroiditis as it accelerates the complete loss of thyroid function

Summary

Thirty five cases of juvenile auto-immune thyroiditis proved by needle biopsies have been evaluated and followed up clinically and studied with radioiodine and other tests of thyroid function as well as by immunological methods. The condition is more frequent than was formerly realized. In some cases it may be diagnosed from the firm character of the goitre and the presence of hypothyroidism, but useful laboratory tests are the 48 hour plasma protein bound radioactivity after administration of I^{131} and antibody tests. Thyroidectomy is contra indicated in children and the treatment of choice is L-thyroxine in full replacement doses. The incidence of mild or severe hypothyroidism at the time of diagnosis is of the order of 20 % but it is probable that progressive thyroid destruction will occur in the majority of patients followed up for many years.

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Erythropoietin Levels in the Amniotic Fluid Particularly in Rh Immunized Pregnancies

by PER HAAVARDSHOLM FINNE

The existence of a humoral erythropoietic regulatory mechanism is now generally accepted. Hypoxia anemic or hypoxic is the primary stimulus for the production of the humoral factor erythropoietin (ESF) which again acts upon the bone marrow. The precise nature site of production and physiological effect of the erythropoietic factor is not quite clear. Probably erythropoietin is mainly produced in the kidneys [5 11 18].

The relationship of erythropoietin to anemia can be elucidated to some extent by measuring the erythropoietin content in plasma [8, 9 14 22]. However there is some difficulty in demonstrating normal or moderately increased erythropoietin levels with the assay methods used today and not all anemic patients are found to have increased amounts of erythropoietin in their plasma [22]. The erythropoietin level and the corresponding anemia shows some difference between patients with various types of anemia [9]. Patients with a hypoplastic marrow tend to show a higher erythropoietin content than those with a hyperactive marrow. This has been taken in favor of the hypothesis that erythropoietin may be utilized by active erythropoietic tissue. There is some disagreement between various investigators concerning the relationship between the increase in erythropoietin level and increasing severity of anemia. Some authors have found an abrupt rise in plasma erythropoietin content when the hemoglobin dropped to less than 5 g [22], but most investigators find increased erythropoietin levels also at higher hemoglobin levels [9].

It has previously been documented that erythropoietin is excreted in the urine [9 15 22] and there seems to be a distinct relationship between the content of erythropoietin in plasma and urine [9 22]. It appears that when erythropoietin is detectable in urine its amount is always lower than simultaneous serum levels suggesting a renal threshold for erythropoietin excretion [9 22].

It is well known that in Rh immunized pregnancies, in spite of greatly increased erythropoiesis, the fetus may suffer hemolytic anemia, in many cases of severe degree. The anemia may be the cause of death either ante or postnatally. The prenatal diagnosis of the severity of the hemolytic disease has assumed increasing importance since it has been shown that

induction of labor before term in selected cases may be essential in reducing the mortality rate from erythroblastosis. Recent studies have shown that since the introduction of exchange transfusions in the treatment of erythroblastosis, the most outstanding problem in hemolytic disease of the newborn is that of stillbirth [21]. This makes it necessary to find indices which can give information regarding the severity of the disease. Maternal antibody titer has been used for a long time, but this does not give much information as to fetal anemia. However it is a useful guide. The bilirubin and protein contents of the amniotic fluid have been proposed as useful guides, but fail in many cases [3, 24] and additional indices therefore would be of value.

Studies on cord blood from normal and anemic infants indicate that the humoral regulatory mechanism of erythropoiesis is intact also during the last part of intrauterine life [ ]. In erythroblastotic anemic infants highly elevated erythropoietin levels have been found in cord blood [7].

There is general agreement that the fetus passes urine into the amniotic fluid. If there is an increased erythropoietin content in the blood this may also reach the amniotic fluid when the erythropoietin level in the blood increases above the suggested renal threshold. Erythropoietin may possibly reach the amniotic fluid by other routes, perhaps through fetal membranes as this way of transport is indicated for other proteins [14].

The purpose of the present paper is to report investigations carried out in an attempt to answer the questions whether anemic *erythroblastotic infants* excrete erythropoietin in the urine, whether erythropoietin occurs in the amniotic fluid in Rh immunized pregnant women, whether erythropoietin is transferred through the urine or through fetal membranes and whether the erythropoietin level in amniotic fluid has any relationship to the anemia of the fetus and the severity of erythroblastosis.

Some preliminary data concerning these problems have been published elsewhere [8].

Materials and Methods

Amniotic fluid from normal and Rh-immunized pregnant women was collected, either by transabdominal amniocentesis before term, or by rupturing the membranes during delivery or during cesarean sections. Blood contaminated samples were discharged. Cord blood and blood withdrawn during the first part of exchange transfusions performed shortly after birth on erythroblastotic infants was collected, as were blood samples withdrawn shortly before delivery from some of the mothers of erythroblastotic infants. The blood samples were centrifuged immediately after collection and the plasma pipetted off. All samples were examined shortly after collecting or frozen and kept at −20°C until studied.

The erythropoietin content in plasma, urine and amniotic fluid was determined using transfusion induced polycythemic mice as recipients, and the Fe^{59} incorporation in red cells as parameter. Six to 8 weeks old white mice were transfused by intraperitoneal injections of packed homologous red cells, with a hematocrit of about 80% on days 1 and 3, according to the directions by Wendell & Rowe [19]. On days 3 and 4 the test material was injected subcutaneously, amniotic fluid and urine in daily doses of 1 ml and plasma in daily doses of 0.5 ml. On day 5 approximately 0.1 microcurie Fe^{59} dissolved in saline was injected intravenously into a tail vein. On day 8 (in a few cases day 7) the mice were anesthetized and blood was withdrawn by heart puncture, pipetted

off int glass tubes and weighed. The tubes were filled with dist lled water to the 3 ml mark in order to hemolyze the red cells. In each mouse the hematocrit was deter mined using the microhematocrit method Each mouse was weighed at the end f the experiment and the blood volume calculated 8% of the body weight. Mice with hema tocrits below 55% were excluded.

A w II type scintillation counter (SC Autoscaler Tracerlab. Inc Boston, Mass. USA) with an efficiency of about 1 10⁶ p.m. per microcurie of I ⁵⁹ at a background f less than 180 c.p.m. was used.

The Fe^{59} incorporation was expressed as the total erythrocyte uptake in per cent f the injected dose and calculated as follows:

Fe^{59} per cent uptake

$$= \frac{\text{Body weight} \times 8 \times \text{sample count} \times 1000}{\text{Sample weight} \times 100 \times \text{counts injected}}$$

In few cases the reticulocyt response curve was determined. The mice were made polycythemic in the same w y as for the Fe^{59} incorporation assay Five days after the last blood injection, when there were no reticulocytes in peripheral blood, the test material was injected in a dose f 2 ml subcutaneously (day 0) and preparations made for reticulocyt counts on days 0, 3 4 and 6. The reticulocytes were stained with Nile blue Tw thousand red cells were counted in each slide In one group f mice 1 5 ml amniotic fluid was injected and reticulocyt counts performed on days 0, 2, 3, 4 and 6.

Results

Table I shows the mean Fe^{59} incorpora tion in 10 polycythemic mice injected with saline and the Fe^{59} uptake in mice injected with amniotic fluid from normal pregnancies. From the table it will be seen that no erythropoietic activity was detec ted in most of the samples, whilst a slight activity was possibly present in two of them.

TABLE 1 *The Fe^{59} per cent erythrocyte uptake of polycythemic mice njected with normal saline and amniotic fluid from nor mal pregnancies*

Test material	Name	Fe^{59} uptake % ± s.e	No. of mice	Mean hema tocrit
Saline		0.6 ± 0.09	19	72
Amniotic fluid	Wl	0.4 ± 0.1	4	74
	Hö	0.9 ± 0.2	3	64
	Lu	0.5 ± 0.1	4	66
	Da	1.2 ± 0 2	5	61
	F	0.7 ± 0.3	5	67
	Sh	0.6 ± 0.3	4	70
	Th	0.5 ± 0.1	4	74
	Le	0.5 ± 0 09	5	73
	H	0.4 ± 0.1	4	70
	Ml	1.2 ± 0.1	4	69

The results of the assays on amniotic fluid from Rh-immunized pregnant women and some clinical data concerning the mothers and infants, are shown in Table 2. These data indicate that erythropoetin is transferred to the amniotic fluid and that the highest erythropoietin levels are found when the fetus is most severely anemic In two cases (Sk³ Bö³) there was no demonstrable erythropoietin in amniotic fluid in spite of severe anemia of the newborn infant. These cases will be discussed later This table show the patient material on which the present and following investigations have been per formed. The names of the patients in this table correspond to those in the other tables.

Table 3 shows the erythropoietin levels in cord blood, amniotic fluid and th first urine passed after birth in some of the cases. The infants hemoglobin and reticulocyte values are also listed. It must be kept in mind that the injected dose of

TABLE 2. *The percentage Fe^{59} erythrocyte uptake of polycythemic mice injected with amniotic fluid from Rh- immunized pregnant women. Some clinical data concerning the mothers and infants are also tabulated*

Name	Anti Rh titre		Cord Hb (g)	Capillary		Gest. age (weeks)	No. of change transf	Fe^{59} uptake %	No. of mice
	Coombs	Albumin		Hb (g)	Ret. ‰				
Pe	1/256	1/256		10.7	160	39	2	34.2±2.8	5
Ol[a]	1/1024	1/2048				38		19.2±0.9	4
Ha	1/512	1/1024	3.5	6.8	310	38	2	14.5±2.4	5
Pr	1/1024	1/512		9.4	41	38	1	10.0±2.4	4
Be	1/128	1/128	4.4	7.5	280	36		4.1±0.8	3
Joh	1/4096	1/4096	6.2	12.4	61	35	2	4.0±0.8	6
J	1/64	1/32	14.1	19.2	33	40	0	3.5±0.9	4
Bö	1/256	1/1[illegible]8	13.2	16.8	50	36	1	2.8±1.8	4
Sk	1/1024	1/512	16.3	19.2	33	38	0	2.7±1.9	4
Gu	1/1024	1/512	8.3			35	3	[illegible]6±2.1	3
HJ	1/512	1/256		12.8		37		2.1±0.5	5
Kv	1/2048	1/512	12.8	16.0	78	37	3	2.3±0.7	5
Chr	1/128	1/128	10.0	14.1		40	1	2.3±0.2	6
Kr	1/[illegible]56	1/128	10.7	14.1	134	40	1	[illegible]0±0.3	5
La	1/512	1/512	14.6	14.8		40	0	1.8±0.[illegible]	6
Lauv	1/1024	1/1024	7.1		139	36	1	1.8±1.2	4
Kro	1/8	1/8	19.2	19.9		40	0	1.4±0.4	4
B	1/2048	1/512	14.6	19.0	50	37	1	1.5±0.7	5
My	1/1024	1/1024		12.8	123	40	3	1.2±0.1	4
Bä	1/1024	1/512	13.2	19.2	1[illegible]0	40	1	1.0±0.3	3
Vl	1/1024	1/128	12.1	14.1	90	40	1	1.0±0.1	5
Ly	1/1024	1/128	14.6	19.9	41	40	1	1.0±0.5	5
Rei	1/1024	1/512	11.7	16.3		38	4	0.9±0.2	5
Joh	1/1024	1/1024		10.3		38	2	0.9±0.2	3
Kl	1/512	1/256	16.3	19.9		40	2	0.6±0.1	4
Bö[b]	1/1024	1/1024	9.7			39	6	0.6±0.2	6
Ny	1/256	1/256	12.1	15.1	45	38	1	0.4±0.1	3
Sk[b]	1/2048	1/512	6.6	7.1	350	37	3	0.4±0.01	3

[a] Died before blood samples were collected.
[b] Meconium discharged before delivery

urine and amniotic fluid was double that of plasma. In one case (Sk[b]) there was a high erythropoietin titer in cord blood and urine but no demonstrable erythropoietin in amniotic fluid. With the exception of this case there is good correlation between the erythropoietin levels in amniotic fluid urine and cord blood

Table 4 shows the erythropoietin content in cord blood and amniotic fluid, and the hemoglobin levels in the infants at birth. It is seen that the highest erythropoietin levels in both cord blood and amniotic fluid were generally found in cases with the lowest hemoglobin levels, but that the erythropoietin content may vary considerably from case to case at the same hemoglobin level.

In 5 cases the erythropoietin content in cord blood, amniotic fluid and maternal blood at birth was determined (Table 5). It is seen that the erythropoietin content in cord blood and amniotic fluid is much higher than in maternal blood.

TABLE 3 *The Fe^{59} per cent erythrocyte uptake of polycythemic mice injected with cord plasma amniotic fluid and urine The hemoglobin values and reticulocyte counts are also listed Numbers in brackets indicate the number of recipient mice The samples are taken from Rh immunized pregnancies*

Name	Cord Hb (g)	Capillary Hb (g)	Capillary Reticulocytes %	Fe^{59} uptake % Cord plasma	Fe^{59} uptake % Amniotic fluid	Fe^{59} uptake % Urine
Ha	2.5	5.8	310	19.3 ± 2.9 (5)	14.1 ± _4 (5)	22.6 ± 2.9 (2)
Be	4.4	7.5	280	2.5 ± 0.2 (8)	4.1 ± 0.6 (3)	_6 ± 0.4 (7)
Sk*	6.6	7.1	350	22.1 ± 2.1 (4)	0.4 ± 0.01 (6)	16.7 ± 1.3 (4)
Lan	1	—	138	6.4 ± 0.1 (4)	1.8 ± 1.2 (4)	0.8 ± 0._ (3)
P	—	10.	—	26.8 ± 3.3 (6)	34.2 ± 3.8 (5)	26.0 ± (1)
Ma	8.3	12.1	123	3.3 ± 0.3 (3)	1.2 ± 0.1 (4)	1.0 ± 0.1 (3)
Kr	10.	14.1	134	13.0 ± 0.3 (3)	_0 ± 0.3 (5)	1.4 ± 0.3 (2)
Ky	1_1	18.1	45	0.4 _ 0.1 (6)	0.4 ± 0._ (3)	0.2 ± 0.0 (4)
Vl	13.1	14.1	90	4.4 ± 0.6 (2)	1.1 ± 0.1 (4)	1.6 ± 0.1 (4)

* Meconium discharged before delivery

Table 6 shows the reduction of erythropoietic activity in amniotic fluid after it had been incubated at 40 C for 20 hours with meconium, compared with another specimen of the same amniotic fluid incubated for the same time but without meconium. Although the numbers of recipient mice are small the data indicate that meconium inhibits the erythropoietic activity of amniotic fluid.

Table 7 shows the erythropoietin content in amniotic fluid from two pregnant women with antibodies persisting from earlier Rh immunized pregnancies, who gave birth to Rh-negative infants in the pregnancies from which the samples were taken

Fig 1 shows the reticulocyte response in polycythemic mice injected with saline amniotic fluid from normal (W) and from Rh immunized pregnant women who gave birth to erythroblastotic anemic infants (Ha, Pr) The figure illustrates that the reticulocytes reach a peak on day 3 (Ha 1.5 ml) and that this peak corresponds to that seen when standard erythropoietin is injected

Fig 2 shows the correlation between the erythropoietin content in cord blood and the hemoglobin level in the infant Fig 3

TABLE 4. The Fe^{59} per cent erythrocyte uptake of polycythemic mice injected with cord plasma and amniotic fluid from Rh immunized pregnant women. Hemoglobin levels and reticulocyte counts are also tabulated. Numbers in brackets indicate number of recipient mice.

Name	Cord Hb (g)	Capillary Hb (g)	Capillary Ret. ‰	Fe^{59} uptake % Cord plasma		Fe^{59} uptake % Amniotic fluid	
Ha	3.5	5.8	310	18.3 ± [illegible]	(5)	14.1 ± [illegible]	(5)
Bm	4.4	7.4	290	3.6 ± 0.3	(6)	4.1 ± 0.6	(5)
Sk*	6.6	1	380	23.1 ± 2.1	(4)	0.4 ± 0.01	(6)
Joh	8.3	12.4	61	8.3 ± 1.1	(7)	4.0 ± 0.8	(6)
Lau	1	—	136	0.4 ± 0.1	(4)	1.8 ± 1.[illegible]	(4)
Pr	—	9.4	41	27.1 ± [illegible]	(4)	10.0 ± 1.0	(4)
Joh	—	10.3	—	[illegible]7 ± 0.2	(4)	0.8 ± 0.1	(3)
Pe	—	10.7	—	46.8 ± 3.3	(6)	34.2 ± 3.8	(3)
Gu	8.3	—	—	15.2 ± 3.7	(4)	[illegible] ± [illegible]	(3)
My	6.3	12.1	123	3.3 ± 0.3	(3)	1.3 ± 0.1	(4)
BöE*	9.7	—	—	23.5 ± 0.7	(4)	0.6 ± 0.2	(3)
Hj	—	12.8	—	1.3 ± 0.3	(5)	[illegible]1 ± 0.2	(3)
Kr	10.[illegible]	14.1	134	16.0 ± 0.3	(3)	[illegible]0 ± 0.2	(3)
Vi	1[illegible]1	14.1	90	4.1 ± 0.6	(6)	1.0 ± 0.1	(3)
Nyb	12.1	15.1	45	0.4 ± 0.1	(6)	0.4 ± 0.1	(3)
Kv	12.2	16.0	8	2.9 ± 1.5	(3)	[illegible]3 ± 0.7	(5)
Bå	12.9	19.[illegible]	129	2.6 ± 0.6	(4)	1.0 ± 0.2	(3)
Bör	13.7	16.8	50	2.4 ± 1.4	(4)	2.8 ± 1.8	(4)
Je	14.6	19.[illegible]	38	10.7 ± 4.7	(3)	3.6 ± 0.9	(4)
Lav	14.6	14.8	—	0.4 ± 0.1	(4)	1.8 ± 0.[illegible]	(3)

* Meconium discharged before delivery

TABLE 5. The Fe^{59} per cent erythrocyte uptake of polycythemic mice injected with amniotic fluid, cord plasma and plasma from the mothers in 5 Rh immunized pregnancies. The hemoglobin levels of the infants and the mothers are also shown. Numbers in brackets indicate number of recipient mice.

Infant	Cord Hb (g)	Cap. Hb (g)	Fe^{59} uptake % Cord plasma	Fe^{59} uptake % Amniotic fluid	Fe^{59} uptake % Mother plasma	Mother Hb (g)
Ha	3.5	5.8	18.3 ± 2.9 (5)	14.1 ± 2.4 (5)	0.8 ± 0.[illegible] (4)	11.5
Sk	4.6	7.1	23.1 ± [illegible] (4)	0.4 ± 0.01 (6)	3.1 ± 0.8 (3)	11.1
Gu	8.3	—	15.2 ± 3.7 (4)	2.6 ± 2.1 (3)	0.7 ± 0.2 (3)	11.1
Ny	12.1	15.1	0.4 ± 0.1 (6)	0.4 ± 0.1 (6)	0.8 ± 0.1 (6)	12.5
Pr	—	9.4	27.1 ± [illegible] (4)	10.0 ± 1.1 (4)	1.1 (1)	11.5

TABLE 6 *The Fe^{59} per cent erythrocyte uptake of polycythemic mice injected with amniotic fluid from a Rh-immunized pregnant woman who gave birth to an anemic infant (Pr) and one who gave birth to a non affected infant (X) To the last sample standard erythropoietin was added (Armour Pharmaceutical Company) in dose of 0.05 ml per ml amniotic fluid To a specimen from both samples meconium was added All samples were incubated for 20 hours at 20°C Numbers in brackets indicate number of recipient mice*

	Fe^{59} % uptake after incubation	
Name	Without meconium	With meconium
Pr	5.3 ± 0.2 (3)	0.8 ± 0.1 (2)
X	20.8 ± 7.1 (5)	3.2 ± 2.9 (2)

demonstrates the relationship between the erythropoietin content in amniotic fluid and the hemoglobin level of the infant The figures are based on the data presented in Tables 2 and 4

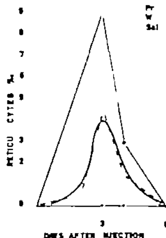


Fig. 1 The reticulocyte response in polycythemic mice injected with amniotic fluid from normal (W) and Rh immunized pregnant women (Pr Ha) Polycythemic mice injected with normal saline were used as controls. Reticulocytes were counted on days 0, 3, 4 and 6 after injection of the test material, but one group of mice had additional reticulocyte count on day 2. The curved line plotted on the figure shows the reticulocyte response curve in mice injected with standard erythropoietin [4].

Discussion

The present investigation has demonstrated the presence of increased erythropoietin levels in cord blood from anemic erythroblastotic infants. Further urine produced antenatally has been found to contain erythropoietin in fetuses which had raised erythropoietin levels in cord blood. The transfer of erythropoietin to the amniotic fluid in pregnancies with anemic erythroblastotic fetuses is also

TABLE 7 *The Fe^{59} per cent erythrocyte uptake of polycythemic mice injected with amniotic fluid from women with persisting Rh-antibodies from earlier Rh-immunized pregnancies, who gave birth to Rh negative infants in the pregnancies from which the samples were taken Husbands were Rh positive heterozygots*

	Anti-Rh titre		Cord	Capillary		Gest.	Fe^{59}
Name	Coombs	Albumen	Hb (g)	Hb (g)	Ret. ‰	age (weeks)	uptake %
MI	1/2048	1/1024	—	16.6		34	1.[illegible] ± 0.1 (4)
F	1/256	1/128	14.7	10.9		40	0.7 ± 0.1 (3)

TABLE 4. *The Fe^{59} per cent erythrocyte uptake of polycythemic mice injected with cord plasma and amniotic fluid from Rh- immunized pregnant women. Hemoglobin level and reticulocyte counts are also tabulated. Numbers in brackets indicate number of recipient mice*

Name	Cord Hb (g)	Capillary Hb (g)	Capillary Ret. ‰	Fe^{59} uptake % Cord plasma		Fe^{59} uptake % Amniotic fluid	
H	3.5	5.8	310	19.3 ± 2.9	(5)	14.1 ± [illegible]	(5)
Be	4.4	7.4	[illegible]90	3.8 ± 0.2	(4)	4.1 ± 0.6	(3)
Sk*	6.6	7.1	380	23.1 ± [illegible]1	(4)	0.4 ± 0.01	(6)
Joh	6.[illegible]	12.4	61	6.3 ± 1.1	(7)	4.0 ± 0.8	(6)
Lan	7.1	—	138	0.4 ± 0.1	(4)	1.8 ± 1.2	(4)
Pr	—	9.4	41	*7.1 ± 2.8	(4)	10.0 ± 1.0	(4)
Job	—	10.3	—	[illegible]7 ± 0.2	(4)	0.8 ± 0.1	(3)
Pe	—	10.7	—	26.8 ± 3.3	(6)	31.[illegible] ± 3.8	(5)
Gu	8.3	—	—	18.3 ± 2.7	(4)	[illegible]8 ± [illegible]1	(2)
My	8.3	12.1	123	3.3 ± 0.3	(3)	1.[illegible] ± 0.1	(4)
BöE*	9.7	—	—	23.5 ± 0.7	(4)	0.6 ± 0.2	(3)
Hj	—	12.8	—	1.2 ± 0.3	(5)	[illegible]1 ± 0.3	(3)
Kr	10.7	14.1	134	16.0 ± 0.3	(3)	[illegible]0 ± 0.2	(5)
Vl	12.1	14.1	90	4.1 ± 0.6	(6)	1.0 ± 0.1	(5)
Nyh	12.1	15.1	45	0.4 ± 0.1	(6)	0.4 ± 0.1	(3)
Kv	12.8	18.0	78	3.9 ± 1.5	(5)	3 ± 0.7	(3)
BA	1[illegible]9	19.2	120	2.6 ± 0.6	(4)	1.0 ± 0.[illegible]	(3)
Bör	13.7	16.8	60	2.4 ± 1.4	(4)	2.8 ± 1.9	(4)
J	14.6	19.2	38	10.7 ± 4.7	(3)	3.5 ± 0.8	(4)
Lav	14.6	14.8	—	0.4 ± 0.1	(4)	1.8 ± 0.[illegible]	(6)

* Meconium discharged before delivery

TABLE 5. *The Fe^{59} per cent erythrocyte uptake of polycythemic mice injected with amniotic fluid, cord plasma and plasma from the mothers in 5 Rh-immunized pregnancies. The hemoglobin levels of the infants and the mothers are also shown. Numbers in brackets indicate number of recipient mice*

Infant	Cord Hb (g)	Cap. Hb (g)	Fe^{59} uptake % Cord plasma	Fe^{59} uptake % Amniotic fluid	Fe^{59} uptake % Mother plasma	Mother Hb (g)
Ha	3.5	5.8	19.3 ± 2.9 (5)	14.1 ± 2.4 (5)	0.8 ± 0.2 (4)	11.5
Sk	6.6	7.1	23.1 ± 2.1 (4)	0.4 ± 0.01 (6)	2.1 ± 0.5 (3)	11.1
Gu	8.3	—	18.3 ± 2.7 (4)	2.8 ± 2.1 (3)	0.7 ± 0.2 (3)	11.1
Ny	12.1	15.1	0.4 ± 0.1 (6)	0.4 ± 0.1 (6)	0.6 ± 0.1 (6)	12.5
Pr	—	9.4	*7.1 ± [illegible]5 (4)	10.0 ± 1.1 (4)	1.1 (1)	11.5

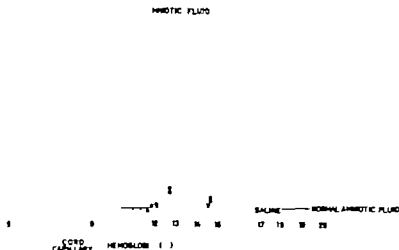


Fig. 3. The Fe[59] per cent erythrocyt uptake in polycythemic mice injected with amniotic fluid from Rh- immunized pregnant women and the corresponding hemoglobin value of the newborn infant (cord or capillary blood). The figure is based on the data presented in Table 2.

Even with a definit anemia it happens that no erythropoietin can be detected by the ssay methods in use today Because f the relationship between the erythropoietin content in cord blood and amniotic fluid, one thus might xpect to find variable erythropoietic activity of mniotic fluid t the same hemoglobin level in the fetus. The fetus may sometimes suffer some degree of anemia when normal or only lightly elevated erythropoietin levels in the amniotic fluid are found ppears from Fig 3 The data concerning the question at which hemoglobin level in the fetus with hemolytic disease one might expect increased erythropoietic activity in the amniotic fluid, are relatively few They indicate however that the amniotic fluid may contain moderately increased amount of erythropoietin at hemoglobin level down to 10–11 *g and greatly in*creased amounts when the hemoglobin level of the fetus drops belo 10 g (Fig 3).

In conclusion one can say that d finitely elevated erythropoietin levels in amniotic fluid indicate a fetal anemia, but modera t ly elevated ...

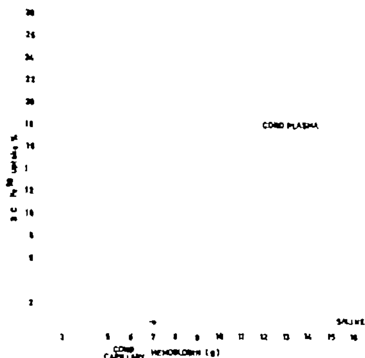


Fig. 2. The Fe[59] per cent erythrocyte uptake in polycythemic mice injected with cord plasma from erythroblastotic infants and the corresponding hemoglobin level in cord or capillary blood. The figure is based on the data presented in Table 4.

documented. Thus, in the fetus, as well as after birth, erythrocyte production is regulated by humoral factors.

Table 1 and Fig. 1 show that amniotic fluid from normal pregnancies contains no or only slight erythropoietic activity as measured with the polycythemic mice method. The slight erythropoietic activity found in some cases fits in well with the observation that small amounts of erythropoietin may be demonstrable in cord plasma from normal pregnancies [7]. This is in accordance with the lively erythropoietic activity of the blood producing organs during fetal life.

There seems to be a good correlation between the erythropoietin levels in cord blood, urine and amniotic fluid (Table 3) and also some correlation between the levels and the anemia of the infants. This means that specimens of amniotic fluid taken ante-natally by amniocentesis may give an idea of the anemia of the fetus in Rh immunized pregnancies.

In amniotic fluid from different pregnancies, variable amounts of erythropoietin are found even when hemoglobin content in fetal blood is the same. Previous investigations in adults on the relationship between the degree of anemia and the corresponding erythropoietin content in the plasma show that one may find marked differences in erythropoietin content in persons having the same hemoglobin level [9, 22]. The differences are more pronounced in hemolytic than in aplastic anemias.

tion, or transfer of erythropoietin from the mother to the fetus, since no or only little erythropoietin was found in blood from mothers in whom the amniotic fluid showed a high erythropoietin content. The erythropoietin found in cord blood and amniotic fluid is of fetal origin. Thi is in agreement with the observations of Jacobson *et al.* [12]. They found that the embryo of the transfusion induced polycythemic mouse had the capacity to initiat and maintain erythropoiesis, even though no erythropoietin seemed to be available for the maternal circulation.

The sit of formation of erythropoietin is not quite clear Some investigations point to the kidneys as the most important erythropoietin producing organ, but it ha also been shown that the kidneys are not the only site of formation [5 10 18]. In fetuses with renal agenesis, there is no anemia at delivery In these infants other organs may probably produce erythropoietin, since the present and other in vestigations [12] seem to indicate that erythropoietin does not pass from the mother to the fetus although one can not entirely exclude the possibility that in these cases maternal erythropoietin may be responsible for the stimulation of fetal erythropoiesis.

The good correlation between the erythropoietin level in cord blood, urine and amniotic fluid indicates that erythropoietin is of the same origin in all these fluids. It must however be remembered that the injected dose of plasma is half that of urine and amniotic fluid. This means that the erythropoietin content in cord blood is always greater than in urine and amniotic fluid, and that the plasma erythropoietin is the source of that in urine and amniotic fluid. This is also in agreement with previous investigations and the suggested renal threshold [9 22]

Fig 1 shows the reticulocyte response in polycythemic mice injected with saline and with amniotic fluid from normal and Rh immunized pregnant women. It is seen that the reticulocyte response is identical to that found when standard erythropoietin is injected, with the peak reached on day 3 and 4 in accordance with the 72 hours Fe^{59} uptake used in the present work, and the increased Fe^{59} uptake corresponds to elevated reticulocyte counts in peripheral blood

Since hypoxia be it anemic or hypoxic is the stimulus for erythropoietin production, and the fetus normally lives in an environment of lower oxygen tension than the infant after birth [17 20] one might also expect slightly increased erythropoietin formation in normal pregnancies. As mentioned before the present and previous investigations [7] on erythropoietin levels in cord blood fits in well with this. However hypoxia during fetal life for reasons other than fetal anemia may cause increased erythropoietin formation. Maternal anemia and placental dysfunction may also exaggerate the low oxygen tension and give rise to measurable erythropoietin content

Previous investigations have hown a reduction in the oxygen carrying capacity of the red cells in erythroblastotic infants, beca se of antibody coating on erythrocytes [1]. This means that the anemic erythroblastotic infant may suffer a more severe anemic hypoxia than the hemoglobin level indicates, and one could expect higher erythropoietin levels in ane

Tables 2 and 3 show that the erythropoietin content in cord blood seems to be higher than in urine and amniotic fluid in specimens obtained simultaneously The plasma dose injected was only half that of urine or amniotic fluid. This supports the view that erythropoietin is excreted to the amniotic fluid through the urine and that there is a renal threshold for excretion. It may be concluded that the higher the erythropoietin level in amniotic fluid the more likely is it that the fetus will be anemic The detection of small amounts of erythropoietin in amniotic fluid from some normal and Rh immunized pregnant women without any fetal anemia indicates a lively erythrocyte production in agreement with the lively erythropoiesis during fetal life In immunized pregnancies without fetal anemia this may probably also indicate an increased rate of erythrocyte production compared with the normal fetus, sufficiently great to compensate for a supposed mild hemolytic ...ase with increased red cell destruc-

The demonstration of increased erythropoietic activity of the amniotic fluid may be of particularly great value in cases where the mother has been immunized during earlier pregnancies, with persisting antibodies in maternal serum and where the father is heterozygous. From a practical point of view these cases are often difficult to handle as illustrated in Table 7 The table shows the data concerning two pregnancies which gave birth to normal non-affected Rh negative infants though greatly elevated antibody titres were demonstrated in maternal serum The corresponding erythropoietin content in amniotic fluid indicates only a normal erythropoietic activity and thus no active hemolytic disease in the fetuses.

In two cases there was not *demonstrable* erythropoietin content in amniotic fluid in spite of severe anemia of the fetuses and highly elevated erythropoietin levels in cord blood and urine (Tables * and 4—Bö* Sk*) In both cases the meconium had been discharged before delivery Probably the meconium with its proteolytic ferments, had destroyed the erythropoietin. Proteolytic activity of the meconium was also demonstrated in gelatine tubes. Table 6 shows that incubation for *0 hours with meconium destroys the erythropoietic activity of amniotic fluid. It is known that trypsin destroys erythropoietin [13], and trypsin in meconium may perhaps account for the lack of erythropoietin in the amniotic fluid

It is not quite clear how the erythropoietin is transferred to the amniotic fluid. However the urine is obviously one of the routes. It is known that the fetus passes urine into the amniotic fluid and it has been shown that fetal urine may contain erythropoietin. There are some reasons for assuming that the placental and cord surface may be actively involved in the passage of protein [23]. It is therefore possible that also erythropoietin may pass from fetal blood to amniotic fluid by this route The presence of erythropoietin in amniotic fluid demonstrates that at least some of the proteins in amniotic fluid are of fetal origin. The source of the amniotic fluids is not clear It seems to be of both fetal and maternal origin. The great amounts of erythropoietin found in amniotic fluid make it seem unlikely that there i any exchange of erythropoietin between the amniotic fluid and maternal circula

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REVIEW ARTICLE

Congenital Hypoplasia of the Exocrine Pancreas

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Introduction

The purpose of this paper is to report two cases of congenital hypoplasia of the exocrine pancreas

These cases together with those that we have collected from the literature offer a distinctive clinical picture of the condition Pancreatic biopsy affords a means of establishing the diagnosis during life

Case 1 G T

This boy was born on the 14th August 1959 H is the only child of healthy parents. ere was nothing of relevance in the family ory

At 9 weeks of age he was admitted to Oldchurch Hospital Essex, because of failure to thri e from the first days of life associated with the passage f frequent offensive motions. In estigations showed absence of tryptic act vity in the stools; a normal gl cose tolerance curve and a specimen of duodenal juice contained no trypsin. It was considered that he had f brocystic disease f the pancreas, and treatment with pancreatin was instituted

At the age of *7 months the boy was admitted to The Hospital for Sick Children Great Ormond Street under the care of R.C.L. on account f diarrhoea with offensive stools, and failure to thrive S nce the original diagnosis he had had occasional respiratory infections, but these had not led to any persistent disease of the lungs.

On admission, his height at 60 cm and weight at 8780 g were both below the third percentile He had slight cl bbing of the fingers. Syndactyly between the 3rd and 4th toes of the right foot was noted, and the helix of each pinna was unfolded The spleen was not palpable The stools were pale greasy and very offensive The appetite as poor partly accounted for by dental caries.

Investigations included the following.

Radiology No persistent pulmonary changes; slight delay in levelopment of some ossific centres.

Blood Haemoglobin 69 %, P.C.V 23 %, reticulocytes 0 4 %, W B.C 4100 per mm³ with polymorphonuclears 9 %, lymphocytes 90 %. Platelets 70,000 per mm. A low platelet count, and a very low proportion of polymorphs in a low total white count proved t be a persistent feature Fuller details of the haematological data throughout the course of the disease re given in Table 1

Bone marrow Slightly h pocellular Of 300 cells counted there were polymorphs, 3 band cells, % metamyelocytes, 4 myelocytes, 4 % promyelocytes, % lymphocytes, 26 % normoblasts, unidentified primitive cells 14 %. The appearances indicated a failure of maturation of granulocytes.

Effect of treatment When pancytopenia with a hypocellular bone marrow had been found several therapeutic agent were tried, including folic acid, intra venous H 1–, anabolic steroid and Prednisone; only the last named seemed to have any beneficial effect on blood cell production (Table 1)

TABLE 1 *Haematological records of Case 1*

Date	Hgb. %	C.I.	P.C.V.	W.B.C.	P	L.	E.	M.	Platelet	Retics
17.11.61	60		32	4100	9	90		1	70,000	0.4
21.11.61	45			3000	6	89		5	66,000	
30.11.61	61			4750	3	89		6	[illegible]	1.1
11.1.62	59			2400	3	89		8	[illegible]	0
19.2.62	66			2500	15	68		17	26,000	
26.2.62	64			2300	23	63		15	34,000	1.4
				Prednisone 10 mg b.d.						
28.5.62	47			4700	6	90			85,000	4.0
4.6.62	54			4600		98			101,000	[illegible]
12.6.62	53			3700	12	83		5	117,000	[illegible]
20.6.62	67			4600	1	70		9	117,000	
10.7.62	63			4400	29	88		11	123,000	5.0
				Prednisone 5 mg b.d.						
[illegible]	64			4000	27	43		30	[illegible]	[illegible]
23.8.62	73			4200	39	51		8	94,000	–
10.9.62	67		33	3850	23	65		1	123,000	1.2
22.10.62	64		33	4200	[illegible]	78		9	120,000	1.8
				Prednisone 15 mg on alternate days						
19.11.62	64		33	3400	14	77		7	80,000	[illegible]
10.12.62	62		34	3400	19	74		7	164,000	1.8
[illegible]	78			3500	22	70		8		
4.1.63	87			2000	49	35		16	120,000	0.8

Serum proteins—total 6.2 g per 100 ml.

Glucose tolerance curve showed a rise of 110 mg% from a fasting level of 73 mg %.

Urine amino-acid pattern normal.

Stool showed no tryptic activity. A 5-day fat balance gave an absorption of 64 %.

Duodenal juice showed no tryptic digestion at a dilution of 1 in 6; lipase 4.8 units/100 ml, amylase 156 Somogyi units/100 ml.

Duodenal biopsy showed a normal mucosa.

Analysis of sweat electrolytes was carried out on two occasions and gave normal results: Na.19 and 26 mEq/l; K.16 and 14 mEq/l; Cl.16 and 27 mEq/l.

He was found to be subject to respiratory and skin infections.

On 27.5.62 a biopsy of the pancreas and of the liver was carried out by Mr Harold Nixon, after consultation of R.C.L. and M.B.

On gross examination there was lipomatosis of the pancreas with sharply defined margins and relatively large lobules. Microscopy revealed a largely fatty stroma in which there were scanty groups of islets of Langerhans with small numbers of pancreatic ducts and a few exocrine acini. The ducts showed no evidence of mucous distension (no mucous).

The liver appeared firm, yellow, fatty and on microscopy mainly perilobular macroglobular fatty infiltration was observed. A slight degree of reticulin fibrosis spread from the portal areas but did not surround the lobules.

Case 2 C.G

This boy was born on May 1st 1955 at full term; birth weight 3060 g. His parents are healthy. The mother is Rh. negative. Her first pregnancy (1947) terminated in a miscarriage at 3 months. A daughter born in

1949 has remained in good health. A boy born in 1950 died at three weeks of anaemia, for which three blood transfusions had been given. He was Rh. positive: a direct Coombs test was negative. The hospital where he died reported that at autopsy the bone marrow was aplastic, but no further details are available. A second daughter born in 1952 has a congenital complete heart block, but is otherwise normal.

Christopher was first seen when 6 weeks old because of anaemia which had been noticed for two weeks. "Physiological" jaundice had lasted for 10 days. On admission the infant was pale and drowsy. The spleen was easily palpable. Investigations showed: Blood group O Rh. negative; haemoglobin 36%; red cells 1.5 million per mm³; W.B.C. 6700/mm³ with polymorphonuclears 13%, lymphocytes 85%. Reticulocytes 11%. Serum bilirubin 0.8 mg per 100 ml. Bone marrow hyperactive with normoblastic erythropoiesis; *there appears to be* a trace of micromyelocytes, and the cells do not develop beyond the metamyelocyte stage. Fragility tests on the infant and his family were normal.

Following transfusion the haemoglobin ... t 114%, but a month later had fallen to 50% rising slowly to 70% under treatment with ferrous gluconate 300 mg daily and folic acid 600 mg daily over a period of 6 weeks. However a low total white count and a poor production of polymorphs, which varied between 2 and 22% persisted through the first two years.

At five months of age the stools were persistently loose and offensive. A fat balance over a 10-day period showed fat absorption to be down to 60%, and examination of the duodenal juice showed complete absence of tryptic activity.

From that date the child has received Pancreatin with his meals.

Duodenal intubation was repeated at nine months and at sixteen months, and on both occasions there was no tryptic activity and lipase and amylase were present only in traces.

The sweat electrolytes were investigated at nine months and at twelve months, and gave normal results (sodium 45 mEq/l and 58 mEq/l, chlorides 44 mEq/l and 54 mEq/l).

Other investigations included the following: gastric secretion: free HCl 28 mEq/l; total HCl 36 mEq/l. A glucose tolerance curve was normal, the blood sugar rising from a fasting level of 93 mg per 100 ml to 123 mg per 100 ml one hour later.

The total serum proteins (at 2½ years of age) were 5.8 g per 100 ml; the electrophoretic pattern of the serum proteins was normal, as was that of the haemoglobin and of the urinary amino-acids.

W. S. and M. B. saw the child together in consultation when he was 18 months old, and considered that in view of the history of severe anaemia at birth, the persistent splenomegaly, the normal sweat electrolytes and the absence of any lung pathology, a diagnosis of fibrocystic disease of the pancreas could not be sustained, although therapy *with pancreatin was clearly indicated.* A biopsy of the pancreas was considered to be necessary for a final diagnosis, but would have to wait until the child's general condition had improved.

The subsequent progress of the child has been one of very slow improvement, often interrupted by respiratory infections chiefly in the upper respiratory passages. These have not given rise to any permanent lung disease and, according to the mother, have been no more frequent in the patient than in his sisters. Following the initial anaemia, the haemoglobin has ranged between ... and 80%. Further haematological data are given in Table 2. The spleen remained palpable until four years of age. Although with pancreatin therapy the fat absorption has risen to between 80 and 90%, and *in the* intervals between respiratory infections the appetite has been at times voracious, growth remains unsatisfactory so that at 6 years of age the height at 112 cm is below the 3rd percentile and the weight at 16.8 kg is at the 5th percentile.

When the boy was 7 years of age he was considered fit enough to undergo a pancreatic biopsy and this was successfully undertaken

Table 2. *Haematological records of Case 2*

Date	Hgb. %	R.B.C.	C.I.	W.B.C.	P %	L. %	E. %	Monos. %	Platelets	Retics. %
1.7.55	96	1.56	1.1	6.7	13	85		2		
4.7.55	116								Ample	
7.7.55	114									
12.7.55	112								Rather scanty	
16.7.55	108				13	75	1	11	Ample	
19.7.55	104									
23.7.55	96									
27.8.55	50			3.7	27	68		3		1.8
3.9.55	60			6.0	7	91		2	Normal	
13.9.55	76			5.8	6	90		4		2
27.9.55	82			7.9	22	78			Normal	
4.10.55	72			7.8	15	84		1		
18.10.55	70			9.4	28	67		1	Normal	
24.10.55	76			7.3	21	63		6		1.5
7.11.55	82			4.6	7	89		4	Scanty	
14.11.55	62			3.0	5	92		3	Normal	0.5
21.11.55	80			4.1	4	95		1		0.5
16.12.55	94			4.6	9	84		7	Normal	
29.12.55	84			6.8	3	96		1		1.7
17.1.56	80			6.2	10	87	1	2	Normal	
1.3.56	60			9.1	12	80		8	Abund.	
5.9.59	77*			7.4	40	52	1	4	Normal	
16.8.63	68			4.9	71	18		10	187 000	1.2

* Aged 4½

by Mr Harold Nixon. The pancreas, on gross examination, was of yellow fatty appearance with sharp margins and large lobules. The liver appeared normal. The pancreatic biopsy consisted mainly of fat, in which there were several isolated but normal islets of Langerhans and lumps of small duct without large ducts. One small collection of pancreatic acini was seen.

Review of the relevant literature

Prior to the publication of our two cases, 18 histologically confirmed cases of this condition have been recorded in the world literature all of whom have died. We shall follow on with references to 18 more children from the literature who are alive and on whom the diagnosis was made on clinical grounds without histological confirmation. Our two present cases are the first instances (to the best of our knowledge) in which the diagnosis has been established histologically during life.

(A) *The histologically confirmed cases*

Case 1

A male aged 12 years, recorded by Opie in 1910 [11], in a moderate nutritional state without steatorrhoea or diabetes, who unfortunately died of septicaemia. At autopsy the pancreas was lipomatous with a few exocrine acini. Islets of Langerhans were present. Hepatic cirrhosis was also present.

Case 2

A male who died at 12 years old of septicaemia, was recorded by Rämle in 1921 [12]. The child had repeated attacks of jaundice infections, and steatorrhoea without evidence of glycosuria. At necropsy the pancreas was lipomatous with small groups of



Fig. 1 Photomicrograph of pancreatic biopsy of Case 1 × 60 showing the lipomatosis and exocrine hypoplasia.

isolated exocrine acini and isolated pancreatic ducts. The islets of Langerhans appeared normal. Fatty infiltration and moderate cirrhosis of the liver were also observed.

Case 3

A male who succumbed to secondary infection at 4 years was reported by Clark & Headfield in 1954 [3]. He suffered from steatorrhoea from the age of 5 months but there was no diabetes. At necropsy there was lipomatosis of the pancreas and fatty infiltration of the liver. No exocrine acini were found but islets of Langerhans appeared to be increased in number.

Case 4

Gross in 1926 [6] reported a male child who died of pneumonia at the age f 19 months. The infant wa marasmic with respiratory infections, steatorrhoea, without diabetes. The clinical diagnosis was coeliac disease At autopsy however lipomatosis f the pancreas was f und with normal islets f Langerhans but pparently complet b- sence f exocrine tissue

Case 5

Hant lmann reported in 1931 [7] a boy who died at 9 years from Hodgkin disease H had no steatorrhoea nor diabetes, but was treated for tuberculous peritonitis, jaundice and Hodgkin disease At autopsy a normally shaped but enlarged and grossly lipomatous pancreas was found which contained rare groups f exocrine acini and normal ppear ing islets. The presence f Hodgkin disease with renal and splenic amyloidosis was con firmed.

Case 6

The child reported by Siwe in 193* [15] died from bronchitis at the age f 2 years. This prematurely born child was marasmic and from six month of age had had severe steatorrhoea with much neutral fat in the stools. Severe ricket was also present. There was no diabetes. At necropsy the enlarged pancreas was lipomatous with rare acinar clumps, with normal islets f Langerhans but without interstitial f brosis. The head f the pancrea was incompletely developed. There was early portal cirrhosis and fatty infiltration f the liver but no emphysema or bronchiectasis were found.

Case 7

Davie [4] in 1938 described the case f a mentally deficient girl who died at the age f 4 years and 4 months. Her stools were never bulky or greasy and she suffered from habitual constipation. She had a voracious appetit During the last two weeks f her life she had had tw ttacks f bronchopneu monia with diarrhoea and vomiting At autopsy she was found t have bronchopneu



Fig. 2. Clinical photograph of Case 2 with control of same age.

monia with numerous abscesses in both lungs, and marked fatty infiltration of the liver The pancreas was somewhat larger than normal and lipomatous. Both main pancreatic ducts were patent. Hist logically the pancreas consisted mainly of adipose tissue in which were embedded a few small groups f exocrine acini and many islets f Langerhans. Pancreatic ducts and branch ducts were present.

Case 8

One f the two cases described by Wissler & Zollinger in 1945 [16]. A boy who gained well t 6 weeks. H had a slight cough at 2 weeks, then developed mucous diarrhoeal stools. Again t 5 weeks he had cough and fever and was admitted to hospital with bronchitis and loss of weight H stayed in hospital for over one year during which time he had recurrent respiratory infections, fre- quent bnormal motions, failure t thrive

and intercurrent erysipelas. He died at 14½ months with the clinical diagnosis of fibrocystic disease of the pancreas. At autopsy there was found chronic interstitial pneumococcal pneumonia with purulent bronchitis and pulmonary atelectasis, but without bronchiectasis. There was hepatic cirrhosis, steatosis and pancreatic lipomatosis, with islets of Langerhans and few remnants of acini. Some fibrous myocarditis was also found.

Case 9

The second case mentioned by Wissler & Zollinger wa a girl with two older healthy siblings. She developed a cough at 4½ months as well as vomiting and diarrhoea. All three children developed pertussis and the girl was admitted to hospital with pneumonia. There the presence of f tty and malodorous stools were observed, for which she was treated. At one year of age she developed another attack of pneumonia and a fortnight later she died with the clinical diagnosis of fibrocystic disease of the pancreas. At autopsy there was bronchopneumonia in both lower lobes, without bronchiect sis. The liver was fatty and the pancreas showed lipomatosis with marked trophy of acini and hyperplasia of the islets of Langerhans, the ducts being normal or increased in number.

Case 10

A 12-year old girl wa labelled Case 3451 Mass. Gen. Hospital 1948 [2], and quoted by Nezelof & Watch in 1961 [10]. She died from pulmonary infection having had repeated ttacks f pneumonia for three years, steatorrhoea from birth, abdominal pain, failure to thri e osteoporosi and retardation of bone development. At topsy there was emphysema, bronchitis, hepatic steatosis biliary cirrhosis, pancreatic lipomatosis with complet trophy f exocrine acini and normal islets of Langerhans.

The present authors ha e a slight reserva tion in accepting this case as a gen ine pancreatic exocrine hypoplasia since the absence of mucosis was not definit ly recorded.

Case 11

Hover [8] in 1949 described a boy who was normal to the age of months. H had virtually doubled his birth weight t months. He developed nasopharyngitis there were bulky malodorous stool with fat floating on the surface. The clinical diagnosi wa of coeliac disease and he was treated accordingly. At one year he aspirated food, collapsed and died. At a topsy the bronchi were obstructed by food remnants and there was oedema of both lungs. The pancreas wa three times the normal weight pale and of normal shape. The xocrine acinar tissue wa completely replaced by fatty tissue. The excreting ducts of the pancreas were normal and so were the islets of Langerhans.

Case 12

Bodian [1] in 195 reported two cases of this condition. The first was a female who died at the age of 10 months. Two blings were well, but two infant had died respe tively at 5 months with gastro-enteritis and at 6 months with pneumonia. The hild was admitted to hospital 5 days bef re her death with marked oedema, a scaly rash, enlarged liver and a marked anaemia (R.B.C ,,30 000, haemoglobin 50%). She had thrived well in the first 6 months but then failed to gain and became pale with frequent malodorous motions. Terminall she received a blood transf sion, developed jaundice and died. At autopsy there was some fatty change of the liver with slight portal f brosis. The l ngs showed patchy collapse and oedema, but no mucosis. Death wa from acute enteritis. The normally sized l pomatous pancreas with large lobules showed groups of islet tissue several main ducts and collecting ductules, but very few exocrine acini.

Case 13

The second case reported by Bodian in 195 [1] was a boy aged 9 years at death. He had bronchopneumonia at two weeks and was in hospital for two months. His motion were always offens and frothy. He failed to thrive and was al mentall backward. A second attack of bronchopneu

monia occurred in the last fortnight before his death, and the clinical diagnosis was fibrocystic disease f the pancreas. At au topsy his brain was unduly small and firm. The lungs showed right-sided bronchopneumonia, no emphysema and no mucosis. The pancreas was large lipomatous, with sharp edges and smooth surfaces. Histologically the organ consisted mainly of adipose tissue in which were scattered small islands f islets of Langerhans with few exocrine acini, normal large ducts but only few ductules. There was no pancreatic fibrosis. Both the main and accessory pancreatic ducts were serially sectioned and found t be freely patent There was minimal fatty change of the liver slight cortical nephrocalcinosis and n evidence of mucosis elsewhere in the body

Case 14

Lumb & Beautyman [9] reported two cases in 195 These were f particular interest as they were siblings. Their first case, a girl, died at 9 months from sulphonamide anuria following a respiratory infection. The child was admitted to hospital aged 1 month, with the diagnosis f fibrocystic disease f the pancreas. She had pneumonia, anaemia, oedema and bulky stools with steatorrhoea. Trypsin was absent both from stools and duodenal juice. At autopsy the pancreas was lipomatous with complet bsence of exocrine acini but normal islets and normal exocrine ducts. Li er and lungs were normal and there was no evidence f mucosis.

Case 15

This little boy (also reported by Lumb & Beautyman [9]) was the second in the same family as Case 14, born 23 months later H was admitted t hospital as a case f imperforat anus with recto-urethral fistula. A colostomy was carried out but he died at 6 days. At autopsy there was no grossly recognisable pancreatic tissue but histological sections revealed the presence of normal pancreatic main ducts, very rare ductules and exocrine acini, some mesen chymal parenchyma with embryonic fat cells and intermediat Laguesse-Benerly communications between islets and acini.

Case 16

Seifert in 1956 [13] recorded a female who died at 19½ months. There was failure t thrive from birth, and fatty stools appeared at 8 months. The child was admitted t hospital with bronchopneumonia and he miplegia and the diagnosis was made of congenital heart disease with cerebral embolism. At autopsy there was lipomatosis f the pancreas with normal islets of Langerhans, hypoplastic exocrine ducts and few exocrine acini without fibromatosis f the pancreas. There was also cardiomegaly diffuse myocardial fibrosis, steatosis and some f brosis f the liver and cerebral softening

Case 17

Finally Nezelof & Watchi [10] reported two more cases in 1961. Their first hild, male, died t 9½ years from monocytic leukaemia. H started with diarrhoea, steatorrhoea, anorexia, vomiting and failure t thrive at * months. The duodenal juice revealed low values of lipase amylase and trypsin. The sweat test yielded normal values. In the absence of pulmonary signs, and since the sweat test was normal, the diagnosis of fibrocystic disease of the pancreas was rejected and that of an isolated pancreatic insufficiency probably due to agenesis of exocrine pancreas was made The hild wa accordingly treated with pancreatic extracts and itamins. At ½ years, however the alimentary symptoms were overshadowed by those due t blood changes. There was a se vere anaemia of 1,170,000 R.B.C" and a neutropenia f 1800. Marrow examination revealed the presence of a leukaemia. Finally the peripheral blood was invaded by monocytic leucoblasts. Remissions were obtained with Cortisone and 6-Mercaptopurine but finally after another two years, the child died with disseminated haemorrhages at 9½ years. At autopsy there was lymphadenopathy hepatomegaly and splenomegaly with widespread leukaemic infiltrations. The enlarged pancreas was entirely lipomatous with adequat numbers of islets of Langer hans and only rudimentary exocrine acini

and ductules without apparent interconnections.

Case 18

The second case recorded by Nezelof & Watchi [10] was of a severely cachectic boy who died at 9 months of cardiac failure. He showed severe f lure to thrive from a few weeks old diarrhoea and anorexia. Severe osteomalacia with several fract ures of ribs developed. Because of a normal sweat test the diagnosis of fibrocystic disease of the pancreas was rejected. There wa a slight hypochromic anaemia with 4,200,000 R B C and 80 haemoglobin as well as a moderat leucocytosis of 10,000 W B.C's with 51% polymorphonuclear leucocytes. One older sister had died at 13 months after a clinically similar syndrome with cachexia and terminal asystole An autopsy was not performed. There was no consanguinity of the parents.

Our present cases constitute Nos. 10 and 20 of all the histologically confirmed cases which we have been able to find in the literature

(B) *Review of the literature of probable cases of exocrine pancreatic hypoplasia without histological confirmation*

Freudenberg [8] in 1934 reported 3 cases.

Case 1

A boy who leveloped diarrhoea, steatorrhoea, fa lure to thri and a tendency to rect l prolapse from the ge f 6 months. Duodenal enzymes were twice estimated and found to be absent. He leveloped fairly well without pancreatic substitution therapy H had no respiratory symptoms and no radiological evidence of pulmonary involvement. The glucose tolerance curv was abnormally high. At 20 ears of age he was relatively w ll with slight igest e trouble and some muscular weakness Mentally he was normal.

Case 2

A 1 months old girl wa admitted to hospital because f fatt bulk stool from early infancy There was a good appetite but abdominal enlargement and f ilure to thriv Three mont previously she suffered transiently from a cough. Duodenal enzymes were absent The glucose tolerance curve was high and there was some glycosuria. She was observed for 4 months There wa no evidence of respiratory inf ction or of bronchiectasis.

Case 3

A ½ year old boy wa seen on account of attacks of bdominal pain and vomiting, bulky and malodorou stools, and pancreatic ach lia except for low values of amylase H was observed f r 4 month and had never shown any respiratory symptoms or signs.

These three children were diagnosed clinically by Freudenberg as instances f *hypoplasia of exocrine pancreas*, and reported by him a such. Histological proof of thi diagnosis was not obtained.

Shwachman, Diamond, Oski & hoo T ik Khaw [14] reported on Pancreatic Insufficiency and Bone Marrow Dysf nction. A New Clinical Entity" at the 27th Annual Meeting of the American Pediatric Society Inc in May 1963, an bstract of which has been published. They here recorded previously unreported syndrome consisting of pancreatic insufficiency failure t thri neutropenia and galactosuria observed in five children, two of whom were siblings. The original diagnosis wa fibrocystic disease of the pancreas, but the lack of p lmonary in olvement and repeated normal sweat test excluded this disease The duodenal fluid showed no activity for trypsin, lipase or am lase but it viscosity was normal. The children, two females and three males ranged in age from 7 months t 9 years. The disease manifested itself linically between and 10 months of lif There wa diarrhoea, failure to gain weigh t pancreatic ach lia and leucopenia a a constant haematological manifestation, being noted on the first day f lif in one patient Bone marrow examinations revealed hypoplasia f all lement in varying degrees, the oldest h ldren showing the most marked hanges. T children had



Fig. 3 Photomicrograph of pancreatic biopsy of Case 60 showing similar features to Case I

thrombocytopenia and in one there was mild anaemia. Serum iron folic acid and vitamin B 12 levels were normal. Galactose was present in the urine in traces, but galactose tolerance curves were normal. None of the patients demonstrated steatorrhoea despite the absence of pancreatic lipase. Duodenal mucosal biopsies revealed normal histological findings. Pancreatic replacement therapy has resulted in satisfactory weight gain only in two of the patients. Therapy with Cortisone vitamin B 12, B 6, folic acid vitamin E, fresh plasma, riboflavine methionine trytophan and pancreatic extract have not corrected the haematological abnormalities.

One patient 15 months old male who has an affected brother aged 6, has in addition to pancreatic insufficiency and neutro-

penia, eczema thrombocytopenia and recurrent infections.

Shwachman and his collaborators state that "the inter-relationship of the pancreatic insufficiency the neutropenia and the galactosuria remains obscure but it seems to the present authors that pancreatic biopsy might well have shown the presence of pancreatic exocrine hypoplasia.

Conclusions

The literature of histologically established cases of congenital pancreatic lipomatosis with hypoplasia of exocrine elements appears to consist of 18 cases, to which we have added another two the first children in whom the diagnosis has been established during life by means of pancreatic biopsy All the other cases had come to autopsy

We have also collected from the literature another 8 cases that clinically correspond to this entity but in them there is no histological confirmation.

The clinical diagnosis is suggested by digestive troubles from early in life comparable to those that occur in fibrocystic disease of the pancreas and the finding of pancreatic achylia The sweat test is, however normal and there are often no respiratory troubles Sometimes however these children have episodes of pneumonia very rarely with any residual emphysema and at autopsy there is no evidence of mucosis.

There is a curious association, which is optional but not obligatory with haematological disturbances of varying severity including anaemia thrombocytopenia and neutropenia associated in some instances with evidence of hypoplasia in the bone marrow of the corresponding cellular elements. The clinical onset of these haematological changes may sometimes date from birth or it may appear later in life.

There is a tendency to a familial incidence in siblings This has been established in two siblings and suspected in two more pairs of siblings. Bearing this in mind, and on account of the early clinical onset of symptoms, and the absence histologically of any evidence of regression or reactive changes to regression in the pancreas, the explanation may perhaps lie in a congenital malformation of the pancreas on a genetic basis This may in some cases be coupled with a relative failure of various bone marrow elements.

The prognosis would appear to be better than that of fibrocystic disease of the pancreas, with which the condition is most likely to be confused.

We would like to emphasise that the decision taken at operation to perform a biopsy should be determined by the presence or absence of a lipomatosis on gross examination We think that a biopsy of such a lipomatous pancreas is without undue danger since the development of a fistula in the absence of enzymes is unlikely

Acknowledgement

We wish to express our thanks to Mr Harold Nixon for performing the biopsies on our two cases, and to Mr Derek Martin and his staff for the illustrations.

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PROCEEDINGS OF PEDIATRIC SOCIETIES

Finnish Pediatric Society

Meeting October 20 1962

C. E. Räihä The possibilities of reducing perinatal mortality

Published in *A Paediat Fen* 9 15–, 1963

M Lelong (Paris, France) Generalized cytomegalic inclusion disease in the newborn infant

Published in *Ann Paediat Fen* 9 113, 1963

Meeting February 23 1963

N Hallm n The need of pediatricians in Finland in the nea future

There re fewer physicians in Finland than in the Scandina ian countries, 1 to 1400 inhabitants. This in licates an obviou shortage of physician in this country There re 115 pediatricians in Finland 50% of *which re men. Sixty of those (43 %) are in* Helsi ki nd only 0 (39%) li e outside the three biggest cities of H lsinki Turku and Tampere

Of the pediatricians ½ are engaged in hospit l work ¼ in preventive medicine and 13 re mainly engaged in privat practice There are t present 0 training post t n versity hospital and the period of req red traini g between 3 to 4 years. In addition to this complet training program is pro ided by the H lsinki City Pediatric Hospital (A rora) nd the biggest of the central hospitals, that t Tampere Partial training can be obt ined t some central hospitals when the number of pediatric bed xceed a certain minimum and the hief pediatrician f lfil req irement of academic standing Of such partial training post there are t present 15. To complete thei pediatric tra ning physicians from these places have to serve for some time t a uni ersity pediatric department Before final certification everybody has to pass an examination.

Under present circumstances 6 to 7 pediatricians q ali fy yearly When the possibilities of the partially training hospital are fully used n additional 5 will qualify yearly At the moment 18 pediatric post are acant and several new ones will be established in the nea future Presumin that as before ½ of pediatricians will go into hospital work these vacant post can be expected to be f lled after bout 5 ears. Considering ll factors the n mber of working pediatricians in Finland in 1980 should be about 250, which would be enough t fulfil the needs of the hospitals but not of the other sectors of pediatrics not at least ith the present scope of trainin ng programs.

I I Määttä Social pediatrics

History The first t exam ne the relationship between society and medicine J P Frank in 1780 J Guérin, in 1848, wa the first to use the concept of social medicine In 191 M Mosse and G Tugendreich published a comprehensi e stud of social medicine Though some work on social pediatrics wa published after the First World War t was not until after the Second World W r that social pediatrics has evol ed as an independen branch of investigation. The f rst study based on social pediatric aspect wa published by F Grundy n 1949

Definition Social pediatrics is concerned with all the environmental factors which influence the health of children and pplies the results of its investigations to further the health f children. Social pediatrics thus includes both the pediatric and social com ponents. In social pediatrics social factors are found to be in causal relationship to the illness f children: social etiology

Methods of investigation. The medical part employs all the methods of medicine which t bottom are based on empirical methods of physics and chemistry The sociological part uses sociological methods like st tistical and scalar measurements. The methods f medicine and sociology are coming closer to each other nowadays be cause medicine employs statistical methods in an increasing degree and sociology strives towards empirical methods of study

Formation of theory: This is done in social pediatrics much in the same way in other branches of science based on empirical research. Intuition provides hypothesis on whi h a plan of investigation is based. Ac cording to this experiments are performed to verify the hypothesis. From the experi ment rule is obtained by way of induction. Rules are formed int theory from which new rules are deduced. These in turn give rise to new experiments. Research thus becomes cumulative each study is linked to a theory and adds to it stability In this way a single study grows in scope

Own investigations: As an example of a study in relation to theory the uthor investigation of social factors affecting hospitalisation f children was shortly reviewed (*An Paediat Fen* 1963)

Importance of social pediatric research in present-day society: Improvement f public health has become an increasingly important goal of social politics. In striving for this public health fficials need the help f social pediatrics t provide them with the factual information on which to base their action in improving the conditions for healthy development f children.

Meeting March 28 1963

E. Rossi (*Bern Switzerland*): Disturbances f protein metabolism in hildhood

Meeting May 11 1963

E. Korkonen Nutriti nal needs of children

R. L. Talkunen Diet and tat f health of rural Finnish children

Results are presented f a field study carried out in rural community of southern Finland where ll the mothers (137) were interviewed and all thei hildren under the age of 15 (307) were examined. The inter view were conducted so as t find out the consumption of various foods within the families and the eating habits of the children as well as the level of social enlightenment and susceptibility to dietary reform of the mothers. Information on consumption re vealed that the calorie intake of this rural population was adequat but the diet wa not aried enough Conspicuous were the bundant use of wheat foods and grain the sparse use of veget bles. When the mean supply of trace lements and vitamins were calculated, it ppeared that the iron supply was barely sufficient for the adult but in sufficient for the children. Also the supply of itamin-C was scarce F ult were found in the eating habit of the children. sparse breakfast consisting mainly of carbohydrates may impaire the effisciency of school age children Even the meals provided at school were one-sided and deficient in calories.

The physical growth f the children w within normal limits. In the few instances where a child was retarded in growth no nutritional causes could be detected. Anemia was frequent: in 46 of preschool-aged and 20 f school-aged hildren. It pre sence was distinctly correlated t bad eating habit and a sparse iron content of the food.

Only half of the children had received vitamin D prophylaxis. In spite of this only 16 showed sign of past or present rickets. In many children who were in a phase of rapid growth and approaching puberty n elevated alkaline phosphatase was found

Finally sociological factors influencing the health of the children were discussed

O Wasz Höckert Nutrition of secondary school pupils

H Kecalainen Iron-deficiency anemia

J Visakorpi Prophylaxis of ricket

To be published in *Duodecim* 1964

Meeting June 8 1963

R. Lagercrantz (Stockholm Sweden) Ulcerous colitis in children and adolescents

During the last 12 years 700 children with ulcerous colitis have been treated or examined at the Children s Clinic of Karolinska Sjukhuset in Stockholm. In a part of these Broberger & Perlmann found both humoral and to lymphocytes bound autoantibodies against ntigen from sterile mucosa of the colon Investigation of the relationship of this antigen to bacterial antigen and the presence of conventional antibodies" in the (usually elevated) serum γ-globulin of the patients is in progress. Allergic symptoms are freq ent in these children and their relat ves, some of the patients are allergic against foods, half of them have had symp ms in organs others than the digestive tract most commonly joints, skin, liver kidney nd mucous membranes. Most of these improve with corticosteroid treatment The disease is therefore regarded rather a an llergic than an inf ctious one possibly an "immunopathy Infection nevertheless probably nfluences the course of the disease primarily as well a secondarily

Medical therapy today is manifold and makes improvement possible in most cases Well-controlled studies ha e proved corticosteroid-treatment valuable in systemic as well as local administration. Acute fulminant cases (about 5% in our material) and chronic invalidating cases with retarded dev lopment (about 15 %) require surgical treatment When the duration of the dis se exceeds about 10 years the risk of a complicating cancer of the colon i high In a selected material of 75 cases 8 developed this cancer an l 7 of them died Thirty fiv of our pati ent ha e had colectomy 10 (with only slight rectal involvement) had an ileorectal anastomosis performed in ad lition the rest a permanent ileostomy which they ha tolerated surprisingly well One patient died in connection with the operation the others are all rehabilitated

S. Öhman A case of mercury poisoning caused by a skin ointment

H Kall ola Hypercalcemia a a cause of dystrophy

I Kantero Helsinki

The Pediatric Society of Southern Sweden

Meeting October 6 1963

S. Widell Neonatal Paroxysmal Tachycardia with Pre-Excitation

A 14-day-old boy who is now 2 years old suddenly became dyspneic and cyanotic Examination revealed pronounced tachycardia. Th s wa soon normal zed and the patient became symptomfree The ECG howed marked P wa es in lead II but w otherwise normal for his ge The patient was given digitalis nd the pulse fell t 0.

At the same time the ECG showed pre-excitation, i.e. shortening f P–Q time nd QRS complex with delta wave This persisted even after the digitalis was discontinued One week later the patient had a new ttack of tachycardia and labial cyanosis. The ECG showed a ventricular rat of 320 and the intervals between the ventricular complex in leads II and III exhibited undulating deformations as in atrial flutter H was gain given digitalis and after a few hours the pulse became normal. The ECG showed pre-excitation as before. The patient received digitalis for 6 months. A soft systolic murmur wa heard now and then in the left I I near the sternum. The size and form of the heart were normal roentgenologically E en after discontinuance of the digitalis the patient remained symptom free except for a brief ttack of tachycardia t 8 months of age This caused only little pallor and exhaustion.

In this case of Wolff–Parkinson–White syndrome the pre-excitation did not appear before the patient had his first attack of paroxysmal tachycardia (p.t.), but thereafter it has remained unchanged. It is known that digitalis facilitates the occurrence of preexcitation, whereas quinidine counteracts this. On account of the high heart rat it is difficult t determine from the ECG whether this is a case f atrial flutter without heart block or p.t of another type Atrial flutter is rare in p.t but more common the younger the patient Prenatally diagnosed p.t. has in more than half f the cases been proven t be atrial fl tter Therapy prognosis and probably also etiology are the same in both conditions.

H Hedeland. Turner Syndrome with Unimpaired Ovarian Function

A 20-year-old girl exhibited many malformations haracteristic of Turner syndrome She had normal menstruation and some development of secondary sexual characteristics after the menarche at 11 years of age From 18 years of age she has had amenorrhea. Hormone analyses of the urine after the onset of amenorrhea showed low total gonadotropin values and estrogen in biologically measurable amounts Antex gonadex tolerance gave very high values of estrogens in the urine determined according to chemical method, indicating that she had functioning ovarian tissue and moreover produced menstruation. The background f her amenorrhea is thus probably hormonal insufficiency t high level for some obscure reason. The patient had been treated bout 5 years ago under the diagnosis f meningoencephalitis. She was judged hromatin positive However chromosome determination revealed 46 chromosomes and normal female karyotype

The question f whether she belongs to the classical Turner syndrome depends on how this is defined. If one means by this women dwarf growth without any known cause and with a varying number f somatic malformations, then among these there are individuals with fully or partially unimpaired ovarian function. In the few such cases described chromosomal aberration has usually been present. However in our patient we cannot completely exclude mosaicism or non identifiable defects in the X chromosomes.

S Widell Idiopathic Hypoglycemia (McQuarrie syndrome)

A twin boy with birth weight f 2479 g who is now 3½ years old, had at 2 and 3 days f age repeated attacks of cyanosis and convulsions, each lasting bout 5 minutes At 10 days of age bleeding was observed in the treum of both eyes. Gradually bilateral impairment in sight developed At 11 months of age the boy had a convulsion in connection with nasopharyngitis with fever The following year similar attacks occurred with intervals of 1–4 months some t the same time as acut infections others without any such connection. The EEG was normal for hi age Treatment with anticonvuls drugs did not have any noticeably prevent e effect against these ttacks. At 2½ years of age the blood sugar during an attack was found to be 0 01 100 ml The fasting blood

sugar during the following days was 0.06. Glucose tolerance and adrenalin test gave normal elevation of the blood sugar. In peroral leucine tolerance test no decrease in the blood sugar occurred. In insulin tolerance test the blood sugar fell from 0.06 g/100 ml to 0.014 g and lay at 0.017 g even after hours. Thus there was probably some relative insufficiency among the normal insulin antagonists in the adrenals.

The patient convulsions occurred mostly in the morning before he had got anything to eat. At bedtime he had quite regularly been given sweets. It seems probable that this increased the risk of post hyperglycemic fall in the blood sugar thereby being a concomitant cause of the convulsions. A protein-rich meal without sweets was prescribed as well as an extra meal immediately upon awakening in the morning. With this regimen the patient has been free from attacks for about one year. The patient's psychic and motor development has been delayed in comparison to that of his twin brother. During the past year some improvement has occurred.

S. Widell: Calcified Cerebral Aneurysm

An 11-year-old boy who for several years had had attacks of nausea and unpleasant sensations of smell became ill with pains over the right eye, nausea, vomiting and a temperature of 38.5 C. Roentgen examination revealed two calcifications in the middle fossa, the larger one about 3 cm. The liquor contained 16 mononuclear cells mm³; after two weeks this number was reduced to 0. The symptoms disappeared after about one week. Encephalography showed that the calcification came from a tumor in the upper anterior part of the temporal lobe. Here the A. cerebri media dexter was occluded. On operation the larger calcification was found deep in the fossa Sylvii. It was considered inextirpable. Toxoplasmosis examination showed on admittance to the hospital dye test 1/50, weeks later it was 1/250. Complement fixation test was negative. Echinococcus test was also negative. Possibly there had been an aneurysm which had ruptured and calcified. The patient was given phenantoin and has been asymptomatic.

S. Widell: Eosinophilic Granuloma

A 1-year-old boy developed in connection with a blow on the right side of the head a swelling of the forehead which lasted for 3 months. Roentgen examination showed destruction here and a similar smaller area on the left side. The skeleton was otherwise quite normal. On biopsy it was shown to be an eosinophilic granuloma. After 2 months the changes had almost completely disappeared.

J. Carlestam: Celiac Disease in Diabetes Mellitus

A 10-year-old girl who had developed diabetes mellitus at [illegible] years of age had attacks of unconsciousness with convulsions. The serum calcium was [illegible] mEq. The girl excreted with the feces 45 g fat/24 hr (normally less than 4 g). Xylose tolerance test showed excretion of [illegible]7 g (normally 4.8 g). Film test showed the presence of trypsin. She was treated with a gluten free relatively low fat diet. The steatorrhea disappeared, but the patient lost weight and the serum calcium remained low. Later bleedings occurred which proved to be due to hypoprothrombinemia. — The girl is now receiving high amounts of unsaturated fat and her general condition is much better than it has been for a long time. The serum calcium has become normal.

Per Selander, Uppsala

Acta Pædiatrica 53 299–300. May 1964

BOOK REVIEW

Jan Lindsten. The Nature and Origin of X Chromosome Aberrations in Turner Syndrome

Almqvist & Wiksell Stockholm, Göteborg Uppsala, 1963 167 p

A baby girl presenting oedema f the feet and perhaps some minor malformations may represent the first indication f a profound biological alteration manifested later in lif as Turner syndrome An examination of the buccal smear will in most cases reveal that the sex chromatin body normally expected in a female is lacking and karyotyping will show the typical XO sex hromosome constitution. In Dr Lindsten material comprising 57 patients with the clinical diagnosis of Turner syndrome, the cytogenetic findings were as mentioned in 35 cases It could be shown by studies f X linked characters that the single X chromosome may be either maternal or paternal in origin. Sex chromatin positivity on the other hand, does by no means indicat normal sex chromosome constit tion, since there is wid variation in type and distri bution f the chromosomal abnormality Mosaicism may be present, indicating disturbances in one or more post-conceptional cell divisions Moreover one X chromosome may be structurally altered. In the monograph three types of such abnormal X chromosomes are described, and a close relationship is shown in the area and DNA content of the sex chromatin body to the size of the abnormal X. In one f the cases no deviation from an pparently normal female karyotype could be demonstrated. This might seem somewhat disturbing at first glance and brings up some problems of definition and selection of patients. Cases of this type may indicat from one aspect that the clinical Turner syndrome and th existence of grossly defective X chromosome pair do not exactly cover each other The converse situation, in which one X is bnormal or lacking in an apparently normal woman with or without menstrual irregularity is one which could perhaps be elucidated by a large scale study on un selected patients, as the author points out. In addition t the many contributions to the cytogenetics f Turner syndrome, Dr Lindsten has ttempted to present as com plet as possible clinical account of his case material. The result is that he has accumu lated information valuable not only for research workers in gynaecological endocrinology but also for clinicians in different fields concerned with this general category of patients. Among these the paediatrician should be particularly familiar with the dif ferent varieties of Turner syndrome and their manifestations in different pre-puberal age groups. In Dr Lindsten monograph the interested paediatrician will find all the necessary information in an enjoy ble logical and scholarly presentation which includes an bundance of excellent illustra tions.

Per Zetterqvist Bromma

Congenital Defects

The International Medical Congress, Ltd Lippincott, Philadelphia and Montreal, 1963 261 pages.

The volume gives a record of the first Inter American conference on congenital defects in 196., with contributions from excellent scientists and clinicians. Improvement in the care of inborn errors is an important problem. Knowledge about their causes and the possibilities f their prevention is still more important A campaign against con genital defects must begin with the study of developmental biology In summarizing

what we know about genetic and structural defects or in other words, how little I known, a conference like this intends to stimulate scientists and clinicians. In this volume the scientific studies of some defects and their clinical manifestations are excellently reviewed. A few clinical papers are of less value Especially the presentations of the molecular basis of genetic defects, abnormal hemoglobins, galactosemia, gargoylism, defects in amino acid metabolism and chromosomal abnormalities are of great interest also to clinicians.

N O Ericson Stockholm

E. Rossi (Ed) Neue Probleme bei Inf k tionskrankheiten des Kindes. S Karger Basel New Yo k, 1964 DM -1 —

This is a report from a Swiss postgrad uate course in pediatrics. Most chapters are written in German. The first one deals with current views on humoral and cellular immunity O Gsell reviews the classificat n I human viral diseases. Other hapters leal with respiratory infections caused by adenovirus alone or associated with bacteria. H Bloch I scusses the best prophylaxis agin t t berculosis. H recommend BCG vaccination; the reviewer i not surprised. The loss of the tuberculin test as a diagnostic tool i once more said to be a serious drawback I general BCG vaccination. Ha this ever seriously endangered a child health? In a chapter on staphylococcal infections m childhood O Tius deals mostly with severe pulmonary infections The epidemiology and other clinical syndromes are hardly mentioned. The concluding two chapters leal with brucellosis and some uncommon infectious diseases of childhood. This book can be recommended for the postgraduat teaching of pediatricians and general practitioners.

Rutger Lagercrantz, Stockholm

ANNOUNCEMENTS

The Second Afro-Asian Congress of Pediatrics

The Second Afro-Asian Congress of Pediatrics will be held in Jakarta, Indonesia, from the 10th to the th of August 1964 All communication regarding the Congress should be addressed t Dr Sutedjo, Pediat ric Department Medical School U iversity of Indonesia, 6, Salemba, J karta, Indonesia.

International Congress of Paediatric Surgeons

The British Association of Paediatric Surgeons will be holding t 11th I ternational Congress under the Presidency of J J Mason Brown, at Rotterdam, the Netherlands, from 31 August to 4 September 1964. All correspondence in connexion w th the C ngress should be addressed t the Secretariat I the 11th B.A.P.S. C ngress Holland Organizing Centre 16 Lange V orhout, The Hague the Netherlands.

Acta Pædiatrica 53, 301–306, July 1964

Institute for the Care of Mother and Child (Pediatric Head, Dr K. Poláček)
Prague Czechoslovakia

The Significance of Blood 'Excess Lactate in the Newborn Period

by K. ZNAMENÁCEK and H. PRIBYLOVÁ

On the basis of investigation of certain parameters of respiratory metabolism in healthy newborn infants during the first 3 days of life [6] we concluded that blood excess lactate" calculated by a method modified from Huckabee [1], represents a suitable criterion for the adequacy of postnatal respiratory metabolism. Similar determinations were carried out in new born infants following complications of pregnancy or delivery and asphyxia, in order to verify the significance of blood excess lactate

Methods

The methods used were those previously described [6]. Determinations f lactat and pyruvat in capillary blood, oxygen consumption, blood glucose and respiratory rat were carried out in several groups, as well as in individual infants from birth to 3 days of age Chief attention was directed to blood lactate, calculated excess lactat and oxygen consumption.

Results

It was confirmed that the level of lactate in cord blood is dependent upon the length of labor nd the further course of blood lactat wa followed in three groups of newborn inf nt differing in manner of delivery nd postnatal linical condition. Six infants born by selective Cesarean section (before onset of uterine contractions) and 10 infants delivered by section on the indication of fetal distress were compared with a control group of 45 infants with normal deliveries (Fig 1).

Blood lactate levels fell relatively rapidly in the control group Lactate in these infants was assumed to be primarily exogenic, transferred transplacentally from the mother Infants delivered by selective section in the course of the first 3 days were never found to have increased lactat levels. In contrast, infants in whom signs of intra-uterine hypoxia led to emergency section tended to have persistently high blood lactate which slowly returned to normal Blood lactate in these infants was taken to represent endogenous sources, resulting from metabolic activity of the hypoxic infant pre and postnatally as would be indicated by the work of Vedra [4].

Direct evidence for the endogenous origin of blood lactate was obtained during the course of two periods of gross hypoxia i a newborn inf nt with a severe irreparable congenital malformation (Fig.) At the height of th asphyctic attack oxygen was given in high concentration. It wa possible to bserve the entire

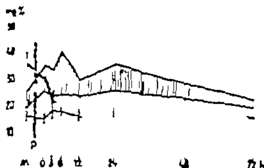


Fig. 1 Lactate level in capillary blood of healthy newborns after normal delivery and pregnancy (thick line), after selective Cesarian section (thin line) and after Cesarian section for fetal distress (dashed area) in the first 3 postnatal days (P = birth). Values from maternal enous blood (M) obtained at the height f the 2nd stage of birth.

course of lactate and pyruvate blood levels with the onset and regression of hypoxia during periods of cyanosis gasping respiration and eventual apnea. The

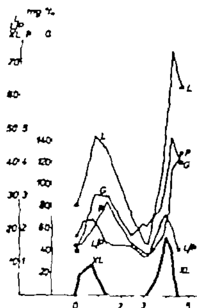


Fig .. The changes in blood lact t (L) glucose (G), pyruvat (P), the lactate: pyruvat ratios (L/P) and excess lact te (XL) during two sphyctic crises in a newborn over the course of 5 hrs. At the height of the crises (max XL conc) oxygen was given by mask.

attacks were terminated by oxygen inhalation. Blood lactate rose to frankly anoxic levels and pyruvate rose but persisted at high levels for some time after oxygen administration and decrease in lactate As can be seen in Fig 2, the absolute level of lactate which even outside the discrete frankly asphyctic episodes tended to be at hypoxic" levels was not in itself in close correlation with the clinical condition while the *L/P* ratio and excess lactate reacted rapidly and *sharply to the hypoxic state and returned* to normal promptly with oxygen a lministration.

It is of interest that in infant born by selective Cesarean section in whom excess lactate was low significantly lower respiratory rates were found (Fig 3)

Comparisons were made between the levels of excess blood lactate and oxygen consumption Observations in three groups of infants with different levels of excess lactate showed that high values of excess lactate were significantly associated with low oxygen consumption. Fig 4 shows three groups of infants with extremes of excess lactate levels: 6 infants born by selective Cesarean section 4 hours after birth 45 infants immediately after normal birth and 60 infants with variou complications of pregnancy and delivery with variou degrees of postnatal hypoxi A similar comparison is shown in Fig 5, which illustrates the time course of excess lactate and oxygen consumption in 10 premature infants (average birth weight "013 *g*). For comparison similar d ta are given for two other groups control infants following normal birth and infant *delivered by selective Cesarean section* Again an inverse relation bet een excess

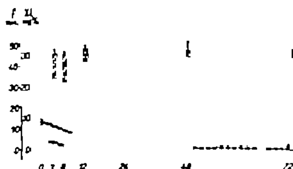


Fig. 3. Changes in lactat in newborns after selective Cesarian section (interrupted line) in comparison with control values (thick line). Respiratory rate of newborns after primary Cesarian section (blank columns) in comparison with the increased respiratory rate in the controls (cross-hatched columns). Period from birth to 3 days.

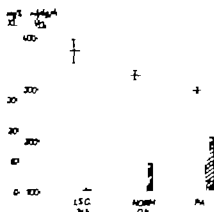


Fig. 4. Oxygen consumption (VO_2, empty columns) and excess lactate (XL, cross-hatched columns) in three groups of newborn 24 hrs after selective Cesarean section (I.s.c.), immediately after normal birth (norm.) and at various times following pathological birth (path.).

lactate and oxygen consumption was found.

The data presented here suggest that excess lactate is a criterion of overall oxygen supply in the newborn and to a certain extent, of the proportion of naerobic metabolism occurring. This conclusion wa confirmed by ttempts to influence the relationship between excess lactat and oxygen consumption by administration of a source of energy in the form of glucose (Fig 6) A group of 10 newborn infants immediately after birth and a second group of 10 infants 48 hours old were given *.5 g of glucose per kg, as 20% glucose intravenously In comparison with control infants receiving no glucose the younger infants showed a significant fall in oxygen consumption and a rise in excess blood-lactate levels.

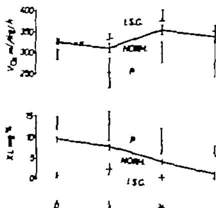


Fig. 5. Oxygen consumption (VO_2) and excess lactate (XL) in the first 3 postnatal days of healthy newborns after normal birth and pregnancy (norm.), after selectiv Cesarian section (I.s.c.) and in prematures after induced labour (P).

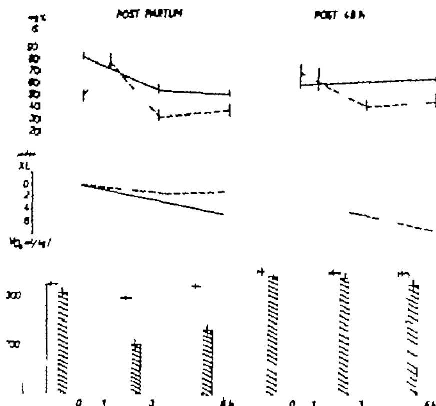


Fig. 6. The relation between blood glucose (G), excess lactate (XL) and O consumption ($\dot{V}O_2$) in newborns just after delivery (left) and at age 48 hrs (right) after i.v. glucose administration.

The older infants on the other hand, showed no change in oxygen consumption and decreases in excess lactate. We conclude that excess lactate reflects the total aerobic and anaerobic processes during the limited period after birth under the influence of maternal metabolism, the birth process, and the intrinsic metabolic process of the fetus and newborn infant.

Discussion

Observations on the significance of excess lactate in the newborn period in relation both to pregnancy and delivery and the condition of the newborn infant have been supplemented by data on the course of the components of excess lactate —blood lactate and pyruvate— and the ratio between the two. We have confirmed the findings of Huckabee that the source of "excess lactate" is a disturbed ratio between lactate and pyruvate occurring under conditions of hypoxia or rather oxygenation of the organism as a whole manifest in a decrease in oxygen consumption. Huckabee maintains that every appearance of an excess of lactate is a reflection of the presence of tissue hypoxia an oxygen debt. Knuttgen [ ] has disagreed with this concept on the grounds that an oxygen debt

can be incurred without lactate accumulation. He concluded that significant lactate accumulation appears only after a certain critical level of hypoxic stress. Knuttgen's work again directs attention to the older concept of the nature of "oxygen debts" as formulated by Margaria, Edwards & Dill [3] in 1933. They divided oxygen-debt manifestations into an alactacid and a lactacid phase, and believed that the latter followed only severe grades of hypoxia, with the appearance of lactate in the blood.

As yet we cannot compare our results with any work dealing with a so-called oxygen debt in the newborn period, as expressed by the appearance of excess lactate in the blood. Our findings indicate that lactate in the newborn may be acquired passively transplacentally and that high levels disappear without difficulty within 3 days of birth. Persistently high levels may be found in the presence of metabolic disturbance or a variety of primary neonatal pathological conditions. Excess lactate may then actively accumulate as a result of tendency to maintain the ratio between lactate and pyruvate (Huckabee). For this reason it has been suggested that blood pyruvate is a more sensitive indication of the trend of carbohydrate metabolism than lactate [8]. We have confirmed Huckabee's opinion that errors inherent in taking lactate alone as a measure of tissue hypoxia are considerably reduced by calculating the "excess" of lactate appearing.

The possibility of differentiating between early and late asphyxia, or rather intra-uterine hypoxia and postnatal respiratory inadequacy has great clinical significance, as does a biochemical indicator of the proportions of aerobiosis and anaerobic glycolysis of carbohydrate metabolism. Procedures designed to influence carbohydrate metabolism, which is probably decisive for the point of view of energy sources in the postnatal period, may be reflected in changes in both components of carbohydrate metabolites, as shown by administration of glucose to new born infants. The important matter of the timing of therapeutic interference with carbohydrate metabolism remains in order to ensure support for the utilization of the energy sources in the neonatal period without aggravating neonatal acidosis.

Summary

The significance of "excess lactate" in the newborn period has been studied, as an indication of overall tissue oxygenation. While blood lactate does not express reliably changes in oxygenation, calculated excess lactate may indicate the proportion between aerobic and anaerobic processes of carbohydrate breakdown and may be used to differentiate between hypoxia of intra-uterine or postnatal origin.

Relationships have been found between excess lactate and oxygen consumption and respiratory rate. Glucose administration was followed by an increase in excess lactate and significant fall in oxygen consumption in the first few hours after birth, but not by the second day, indicating that the time after birth influences the relative roles of aerobic and anaerobic metabolic processes in the newborn period.

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Studies of Urinary Tract Infections in Infancy and Childhood

II Quantitative Estimation of Bacteriuria in Unselected Neonates with Special Reference to the Occurrence of Asymptomatic Infections

by K. LINCOLN and J WINBERG

Urinary tract infections appearing during the first week of life may if untreated, have a serious prognosis [3, 4 5 9 10 12]. In spite of this the interest in the neonatal form of this disease has been little judging from the number of papers, which are few compared to the overwhelming literature concerning infections appearing later in life Swedish experiences suggest that the diagnosis is made fairly seldom. Thus in 1961 the compulsory annual report to the National Board of Health concerning diseases of the newborn included only seven cases of urinary tract infections (those of the present study excluded) among the 70,000 newborns which were under direct paediatric supervision in maternity hospitals. This low incidence of diagnosis—0 1% of all live births—may be due to the fact that the disease is rare Another explanation may be that it is often overlooked. Some observations ctually speak in favour of the latter explanation Thus after scattered reports by different authors in the twenties [5 1*] Craig 1935 [3] was able to collect 61 cases of his own and in 1959 James [6] reported 32 infants less than 2 weeks old with urinary tract infection, an incidence of 1.5% of all live births in the hospital during the period under review Kenny Medearis, Drachman, Gibson & Klein [8] have reported on an epidemical outbreak of pyelonephritis in male infants 2–7 weeks old. Furthermore Stansfeld [14] in 156 children with pyelonephritis found the age of onset of symptoms to be under one year in 90 cases and under one month in 20 cases. The personal observation of a couple of patients acutely ill with pyelonephritis for the first time at the age of some months, although a lifelong history of vagu symptoms suggested a neonatal origin of the disease still more increased the suspicion of a not infrequent overlooking of the disease in neonates.

Because of these experiences a thorough search for urinary tract infections was started in a maternity hospital with the aim of obtaining a material permitting a lose description of the spectrum of the clinical characteristics of the neonatal

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infection. Thus the urinary excretion of cells and bacteria was estimated in patients showing even the slightest symptoms that might have been due to infections. In spite of a good bacteriological technique we got a heavy growth of bacteria in so many urines that it seemed improbable to assume the presence of a urinary tract infection in all these patients. It was thus obvious that a study of neonatal pyelonephritis had to begin with a systematic study of bacteriuria in a material of unselected neonates to define the limits of significant and unsignificant bacteriuria in this age group

The results reported in this paper indicate that unless special cleaning measures of the genital tract are undertaken the values for pathologic bacteriuria given by Kass [ ] and by Pryles Lüders & Alkan [11] cannot be applied to neonates The urinary cell counts in the normal neonate will be dealt with in a following paper Most interesting was however the demonstration of urinary tract infections in 8 of about three hundred unselected male neonates whereas no girl of about 300 seemed to be affected.

Material

Almost 100 of the deliveries in Gothenburg t ke place in maternity hospitals. There was no special accumulation of complicated deliveries in the hospit l where the investigation took place

The material consisted of 584 neonates 298 boys and 86 girls. The patients were strictly selected at random. Prematures and patients with severe asphyxia or sible malformations were, however xcluded A pilot study of 40 patients was performed during the period February–March 1960 The other patients were born during the periods December 1960–March 1961 and September 1961–January 1962.

The urine was examined one or several times during the first seven days of life when the child was in the hospital. In some patients urine was examined *also* during the days 8–15

Methods

I Bacteriological methods

All urine samples were brought to the laboratory in screw capped 10 ml test tubes placed in containers with ice cubes and cultured within 8 hours after voiding Serial tenf ld dilutions were made in sterile saline with 1% meat extract broth. One-tenth ml of urine and dilutions were spread on the whole surface of each of a blood agar plate a lactose bromthymolblue (*Drigalski*) agar plate and a sodium azide sorbitol bromthymolblue agar plat After drying, the plates were incubated aerobically at 37 C for 20 hours read reincubated for another 24 hours and read finally two days after seeding with the aid of Quebec colony counter and a mechanical counting device The three different solid media permitted a good quantitative estimation f the number of each bacterial species present in the urine *During one* period of the investigation the blood agar plates were incubated i closed plastic jars, and an additional blood agar *plate was streaked with a standard* loop of urine and incubated anaerobically for five days.

The term coliform bacteria, or coliforms, were used for all gramnegative large rods growing well on Drigalski agar which either fermented lactose rapidly r were shown not to belong t the groups Salmonella, Arizona, Shigella, Proteus Providencia, Pseudomonas r Alcaligenes It includes Escherichia, Citrobacter Klebsiella and Cloaca (Aerobacter) Eight strains of coliform bacteria isolated from cases with probable infection were serologically investigated at the *Escherichia Center of the Statens Seruminstitut* Copenhagen.

The leucocytes and other formed elements in the urine were counted in uncentrifuged urine in a Fuchs Rosenthal counting chamber

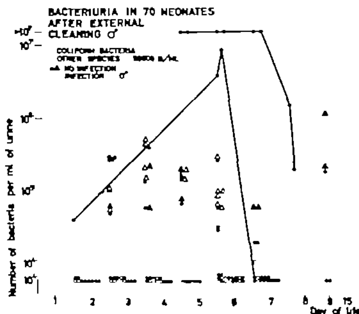


Fig 1 Bacterial counts in 123 urine specimens from 70 males during the first 15 days of life after external genital cleaning with soap and water For each urine sample the coliform count is indicated by one circle Other species are indicated by triangles, when the count was 50,000 or more bacteria per ml.

A bacterial count of 100,000 or more bacteria per ml of urine was found in 43 specimens from 24 boys. Only in two patients the bacteriuria was accompanied by leucocyturia.

○● Coliform bacteria.
△▲ = Other bacterial species when 50,000 bacteria per ml.
○△ Bacteriuria accompanied by unsignificant leucocyturia.
●▲ Bacteriuria accompanied by marked leucocyturia.

II Clinical methods

Patient Group A Before attaching sterile glass container to the infant the genital tract and surrounding area of the skin was thoroughly cleaned with an abundance of soap and water and then dried by a piece of gauze

Patients Group B Two urine specimens were saved, the first one after cleaning described under A, the second one after irrigation of praeputium and vulva respectively two times with 5 ml of physiologic saline each time Also the irrigation fluid was saved for culture

Patients Group C Boys The praeputial sack was irrigated twice with 10 ml of 5 lukewarm solution of soap, gentle massage of the praeputium was performed followed by thorough cleaning of the whole genital tract as described under A Then the praeputium was again irrigated four times with 10 ml of sterile lukewarm water each time At last the whole genital tract was washed with water and dried with a pad of gauze

In the girls corresponding irrigation and cleaning was performed, placing the tip of the syringe near the clitoris. Special care was taken to irrigate the folds between minor and major labia.

After the cleaning procedure sterile glass container was fitted to the patient who was then watched continuously Usually immediately after voiding but not more than 15 minutes thereafter the urine was transferred from the glass container to a

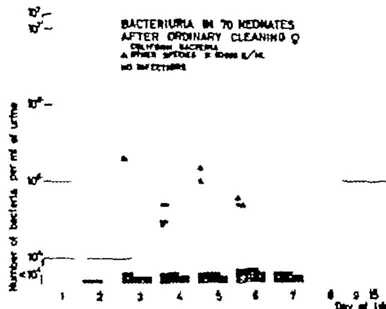


Fig. — Bacterial counts in 12* specimens from 70 females during the first 8 days of life; external genital cleaning with soap and water (Symbol as in Fig. 1)

A bacterial count of 100,000 or more bacteria per ml of urine was found in 5 specimens from 5 girls, none accompanied by leucocyturia.

sterile screw-capped 10 ml tube by means of a sterile syringe and needle. The tube was immediately placed in a jar with ice cubes in which it was transported to the laboratory. If the container tied to the patient was soiled by faeces the specimen was discarded.

Results

Group A Seventy males and 70 females. Urine obtained after thorough cleaning of the genital tract with soap and water.

One hundred and twenty three specimens were obtained from 0 boys and 132 specimens from 70 girls. A bacterial count of 100 000 per ml or more was found in 43 specimens from 24 boys and in 5 specimens from 5 girls (Fig. 1 and Fig.) The strains of bacteria observed in these urines were—except coliforms—enterococci and staphylococci (coagulase positive and negative).

With standards used in adults [7] and older children [11] a bacterial count of 100 000 bact per ml would suggest a urinary tract infection. It seemed, however, improbable that about one third of apparently healthy newborn males would have a urinary tract infection. Since there was a marked difference between boys and girls as regards the frequency of high bacterial counts, an error of the bacteriological technique could be excluded. The apparent sex difference suggested that the bacteria might have originated from the preputial sacks formed by the attachment of the prepuce to the glans and not amenable to routine methods of cleaning.

In two boys only (black symbols, Fig. 1) the bacteriuria was accompanied by a marked leucocyturia, Table 1 (Cases 1 and –). There were in these two patients

Table 1 *Patients with bacteruria and leucocyturia.*

N	Patient		Day of lif	Type of bacteria	Bact. per ml of urine in millions	Leucocytes per mm³ uncent rifuged urine	Symptoms during the first 7 days of lif
1	600211 R.	♂	6	E. coli 046:H32	10	1,000	Slightly pale one day
2	600303 O	♂	5	E. coli 0.4:H5	100	2,000	Slight weight instability
3	601115 D	♂	7	E. coli (069) (0129) (0135):H6	2	300	Crying. Otherwise none
4	601119 K.	♂	6	E. coli Neg (01–0146):H neg*	10	2,000	None
5	600107 W	♂	7	E. coli Neg (01–0146):H6	10	150	None
6	610207 G	♂	7	E. coli 06:H1	100	600	Slight weight instability
7	610317 O	♂	8	E. coli 05:H4	2	100	None
8	611129 E.		7	E. coli	50	70	None
				Enterococci	1		
			8	E. coli (07) 039:H neg	100	20,000	
9	611118 O	♀	6	Alfa-streptococci	0.2	600	None
			7	Enterococci	0.2	175	

Neg (01–0146): the O-antigen did not belong to any of the known 146 O-types.

no or only slight symptoms, the significance of which are difficult to evaluate for the moment

Group B Six males and four females.

To test the above hypothesis urine was collected after ordinary cleaning After this the praeputial sack was irrigated two times with 5 ml of physiological saline each time The irrigation fluid was collected and cultured. After this procedure urine was collected again and new urine culture performed. The same procedure was employed in the girls, placing the tip of the syringe at the base f clitoris. The results are given in T bles 2 and 3 These studies indicated that irrigation of the praeputial sack would reduce considerably the risk of contamination of the urine by praeputial bacteria. Also in girls irrigation seemed to reduce the risk for cont mination

Group C Two hundred and twenty eight males and 216 females Urine obtained after cleaning of the genital tract and irrigation of praepuce or vulvar region as described in methods.

After the initial studies a total of 444 neonates were investigated with regard to

Table 2. *Effect of praeputial irrigation in six patients*

Day of lif	No. of bacteria per ml of Urine: Before irrig.	Urine: After irrig.	Irrigation fluid: I	Irrigation fluid: II
3	240,000	8,000	500,000	300
4	100,000	50	100,000	40,000
5	350 000	2,000	180,000	14,000
5	200,000	2,400	1,500,000	130,000
6	90,000	8,000	3,000,000	120,000
6	< 10	500	60,000	[illegible]

Table 3 *Effect of vulvar irrigation in four patients*

Day of life	No. of bacteria per ml of: Urine, Before irrig	After irrig	Irrigation fluid I	II
3	4,000	< 100	3,000	600
5	100,000	< 100	100,000	5,000
5	1,300	< 50	1,000	100
6	240,000	600	170,000	700
6	10,000	100	5,000	1,500
	2,000	No culture	500,000	5,000
	15,000	< 10	3,000	< 100

the bacterial count of the urine to get an idea of the normal values in this age. Three hundred and ninety four specimens were obtained from 228 boys and 335 specimens from 210 girls. A bacterial count of 100 000 or more per ml of urine was found in 27 specimens from 19 boys (Fig 3) and in 5 specimens from four girls. The types of bacteria excreted are given in Fig 3 and 4. All patients with more than 50 000 coliforms per ml urine and absence of leucocyturia, were checked repeatedly during the first few weeks of life. The bacteriuria vanished spontaneously in all. Leucocyturia exceeding 25 per mm³ uncentrifuged urine and usually reaching very high values was found in 5 boys with bacteriuria (coliuria in all) black symbols, Fig. 3 and Table 1. Cases 3–8. One of the girls with pronounced bacteriuria—excreting *alfa-streptococci* and *enterococci*—had a leucocyturia exceeding 50/mm³ of uncentrifuged urine (Fig. 4 and Table 1 Case 9). Infection was judged as questionable in the girl but highly probable in the six boys, although they had no or only

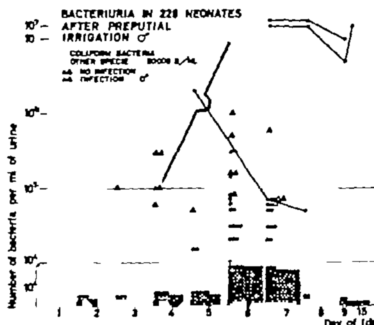


Fig. 3. Bacterial count in 394 specimens from 228 males during the first 15 days of life. External genital cleaning and preputial irrigation with soap and water. (Symbols as in Fig. 1.)
A bacterial count of 100,000 bacteria per ml or more was found in 27 specimens from 19 boys. In six boys bacteriuria was accompanied by leucocyturia.

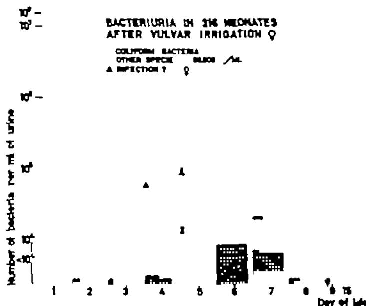


Fig. 4 Bacterial counts in 333 specimens from 216 females during the first 15 days of life Ex ternal genital cleaning and vulvar irrigation with soap and water
▲ = Questionable infection. (Symbols otherwise as in Fig 1)
A bacterial count of 100,000 bacteria per ml or more was found in 4 specimens from 4 girls, only in one accompanied by leucocyturia

questionable symptoms pointing to an infection.

The eight patients with a probable infection (Table 1) will be presented in detail in a forthcoming paper on neonatal pyelonephritis. Suffice it to say here that there were no indications of obstructive malformations and that in some of the patients the urinary findings disappeared spontaneously while in others they persisted until treatment was instituted. In two untreated patients definite symptoms of infection ppeared t the age of 6 weeks and 1 weeks respect vely Urine culture wa found to be positive in both Serological typing howed the bacteria isolated at onset of symptoms t be of th same type ((060)(0120)(0133) H6) as tho-e isolated in the neonatal period, in one of the patients (Case 3 T ble 1) In the other (Case 5) both strains had an O-antigen that did not belong to any of the known 146-types, while the H-antigen was identical (neg (01-0146) H8) (Table 1) Thus also in this case it is possible that it was the neonatal infection that persisted at onset of symptoms with the age of 6 week Infection was rapidly eradicated with treatment in both cases and repeated cultures and sediments during the first year of life were negative It is of interest that an intravenous urography performed at the age of one year in Case 5 showed the left kidney to be markedly smaller than the right one

Comment

The introduction of quantitative estimation of bacteriuria has greatly increased the accuracy of diagnosis of urinary tract infections. When this technique was applied to a study of neonatal pyelonephritis it was soon found that normal values for adults and children were not applicable to neonates, especially not to boys where very high bacterial counts were often found without a concomitant pathologic leucocyturia. It has been shown in this investigation that the praeputial folds, not amenable to ordinary cleaning in neonates may harbour great quantities of bacteria. These can be washed out by the urinary stream and may cause a bacterial count in the urine which by adult standards would be judged as significant for a urinary tract infection. The investigation has shown that this error can be overcome by praeputial irrigation. In boys such an irrigation seems to be necessary before any valid conclusions can be drawn from a bacteriuria between 100 000 and 1 million bacteria per ml In girls ordinary cleaning of the vulvar region with soap and water reduces the risk of contamination of the urine by the vulvar flora considerably. Irrigation of the vulva seems to reduce this risk still further. When irrigation of the praeputial sack or vulvar region was performed before the collection of urine the bacterial counts were of the same magnitude as in adults or children.

The most interesting finding in this investigation was that among 208 boys selected at random there were 8 with a combination of pronounced leucocyturia and colluria. With all probability a diagnosis of urinary tract infection is justified in these patients. In 286 girls there were no such cases with the exception of a questionable one. Since there were 2 infections in the 70 nonirrigated patients and 6 infections in the 228 irrigated ones there is no indication that infection was introduced into the urinary tract by the irrigation procedure.

The preponderance of males has been observed also in other series of pyelonephritis and/or sepsis during early life [1 8, 10 13]. This sex difference is not well understood, but seems not to be explained on the basis of the sex difference in frequency of obstructive malformations [1 8 10 13].

The nature of the disease in the present material is uncertain. Hypothetically it may be due to a bacteraemia. This would be in accordance with the observations by Kenny *et al* [8] who found a positive blood culture in 8 out of 11 small infants with symptomatic urinary tract infections. The eight infections of our series were caused by 6 or possibly 7 different strains of *E coli*. Thus there was no suspicion of an epidemic caused by a special nephropathogenic strain, as suggested in another investigation [8].

Newborns with pyelonephritis will be discussed in detail in a forthcoming paper. Here will only be mentioned that the investigation suggests that the infection may be completely asymptomatic and that infections which give the first clinical symptoms during the first few months of life may have originated in the neonatal period.

The demonstration of a unilateral small kidney one year after the infection in one of the patients leaves the question open whether positive urinary findings in

asymptomatic neonates may signal a chronic pyelonephritis, leading to kidney contraction.

With regard to the high frequency of asymptomatic infections in this study and the possible serious significance of neonatal infections [3 4 5 8 9 10 12 13] the question may be raised whether a routine examination of the newborn's urine would be recommendable

Summary

The urinary bacterial excretion was determined in 584 unselected neonates after different kinds of external genital cleaning. The investigation has shown that after ordinary cleaning of the external genitals 4 of 70 males and 5 of 70 females showed a bacterial count of 100 000 bacteria/ml or more.

The praeputial folds and, to a lesser extent, the vulvar region were shown to harbour great quantities of bacteria causing significant contamination of the urine even after thorough external cleaning. This error can be overcome by irrigation of the praeputial folds or vulvar region. When this measure was undertaken the bacterial counts were of the same magnitude as in children or adults.

The most interesting finding was that among 298 boys selected at random there were 8 with a probable infection, while there in 286 girls were none with one possible exception. In one of the patients in whom infection persisted for six weeks a unilateral small kidney was demonstrated at the ge of one year Whether this was the result of congenital malformation or infection is open to discussion.

Acknowledgements

The thors are indebted to Drs Frit and Ida Orskov International Escherichia Center Statens Seruminstitut Copenhagen, for serological typing f bacteria.

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Artificial Placentae[1]

A Progress Report

by L. LAWN and R. A. McCANCE

Lawn & McCance [14] described an p- paratus which they proposed to develop as an artifical placenta. Before that time there had been two publications on the preservation of mammalian foetuses out side the body in a viable state [19 20] but neither of these preparations seemed to be suitable for physiological work. Since 1962 a group at Edmonton in Canada has also been trying to develop an artificial placenta to use for resuscitation or in respiratory distress [2 3 4]. An unborn lamb has been successfully delivered by these uthors after being maintained for 40 minutes in their apparatus Since then Nixon, Britton & Alexander [16] have described an pparatus by which 3 full term foetal lambs were maintained alive without pul monary respiration for bout 60 minutes on the bench and subsequently delivered.

In the present studies pig foetuses were used.

Apparatus and Methods

Place ta "A"

The original apparatus, now designated placenta A consisted f due oxy genator or "gas-exchanger" which was pri med with about 250 ml f blood from the mother or another sow To this the foetal heart pumped the blood against gravity through cannulae inserted int the two um bilical arteries. The blood was returned to the umbilical vein by gravity through a dialysing system, but this was not essential for periods of survival up t 7 or 8 hours and was often omitted. The arterial and venous flow rates were monitored by drop counters.

Although it was possible t keep pig foetuses alive for 6 to 9 hours with placenta "A" even without a dialyser the apparatus had many physiological and technical shortcomings. The arterial and venous pressures for ex ample had to be set at arbitrary levels for the best ones were not known. The blood in the gas exchanger was directly exposed to the atmosphere or the selected gas mixture and it was difficult to keep the blood in it and the return t bing at the correct physiological temperature The drop counters, more over recorded accurately enough flow rates up t 40 ml/min but not beyond. Further more changes in the flow rat could only be detected during an experiment by counting the dots on the tracing, and fter the experiment was over a considerable amount of work had to be done to make continuous record f the flow A new flow meter based upon better principles has now been construc ted and a description of it is in press [18].

In almost all the experiment the foetus deteriorated in a characteristic way and the

[1] This article is based upon lecture presented at joint meeting of the Danish Society for Gynaecology & Obstetrics and the Danish Society for Clinical Chemistry and Clinical Physiology Copenhagen, September 12th, 1963.

cause of death was usually the same When first set up after cannulation the foetus looked perfectly normal. It often displayed considerable activity and usually did if it was made anoxic. As time went on small ecchymoses appeared here and there and the foetus gradually became more and more congested until finally the whole venous and capillary system became full of blood. In spite of this the output of the heart began to fall and after death the heart muscle was as congested as the rest of the body In spit of the value of this placenta it was decided to make another one which would provide for th gas exchange and if possible dialysis in a different way

Placenta B

The blood in the umbilical arteries is pumped by the foetal heart to the gas exchanger as before but in it there is no direct contact between the blood and the gas mixture The extra-corporeal circulation is entirely enclosed and the blood is not returned to the foetus by gravity or by an external pump. In this system the gas exchange must take place through some material which is permeable to oxygen and carbon dioxide but not permeable to red blood cells Several membranes have been investigated for this purpose Cellophane is slightly permeable to oxygen Teflon or P T F E. is more so and silicone rubber membranes are more permeable still, but unfortunately the latter membranes have been found to be very prone t pin hole in ha. It was found t be dif ficult t support any of these membranes in such a way that the resistance t the flow was low and constant and to ensure that a change f gas pressure on one side did not affect the blood volume on the ther They have now been replaced by rigid plates made of sintered nickel The characteristics f these plates can be controlled but the ones we use are not yet on the market They are about 1 .5 15 cm with an effective area of about 110 cm a porosity f 40 and a mean pore size of 2.5 μ The porosity is a measure of the space between the metal particles, and the "pore size" of the diameter f these spaces. Since the blood corpuscles have a mean diameter of 6–7 μ they do not pass through the plates. The plates are however permeable to gases and liquids and in fact to particles whose size does not exceed 3 μ The amount of oxygen taken up by the blood can be controlled t some extent by varying the pressure of the gas mixture but if this is made too high bubbles form in the blood stream and have t be collected in a trap. The original exchanger consisted of a pack of 8 plates in 4 pairs. The plates in each pair were separated by 1 60 mm and through this space the blood irculated freely Each pair was separated by 2.5 mm and through this space the gas mixture passed The whole pack, which was mounted with the plates vertical, rotated on a horizontal axis through 360 and then returned to the starting position. This kept the blood moving on the plates and prevented any sedimentation. This gas exchanger had a total effective plate rea of 880 cm^2 and through this it was possible to pass about 0.8 ml f oxygen per minute without bubble formation. A 80 day foetus requires rather more than this and we could only keep larger ones oxygenated by raising the O pressure and trapping the bubbles A full description of this apparatus as modified and enlarged is being prepared.

Since the extracorporeal circulation is entirely enclosed in placenta "B only the blood lea ing the foetus can return to it and all f it must do so The rat f the circulation is determined by the activity of the foetal heart, the systemic blood pressure of the foetus and the resistance of the exchanger but this last can be varied either on the arterial or the venous side by screw clip and the pressures recorded on manometers

The pressure flow arrangements pla centa B are quit different from those in placenta A In particular (1) the olume f blood outside the body is much smaller and t is fixed, (2) the enous return does not depend upon a force f gra ity arbitra ril fixed by the invest gat r (3) it is not

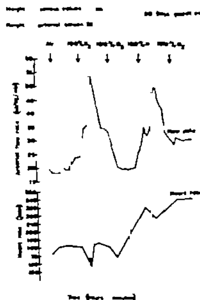


Fig. 1

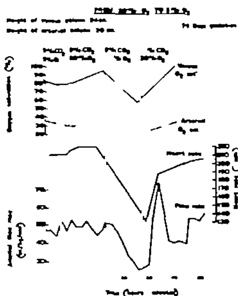


Fig 2

possible for the foetus t become slowly con gested by the imperceptible additions f blood from the exchanger t the venous side f the heart

In consequence of these and possibly other characteristics, the experiments with pla cent B have tended t take a different course from those with placenta A and *the results so far obtained with it will be* described fter those for placent A

Results and Comments

Placenta A"

Fig 1 shows one of the earlier xperi ments when *the recording technique* was rather limited. The gas mixture wa changed from air to nitrogen then through oxygen to nitrogen again and finally back to oxygen. On both occasions on which it w mad the change to N more than doubled the flow rate in the umbilical cir culation nd this reverted to it original rate in the interval. The pulse rate did not change appreciably when N_2 was substituted for air and was rising to a new plateau before during and after the second exposure to N_2. This figure show that the change in the gas mixture took some minutes to produce its results and the peak of the response was not observed till after the mixture had been changed again. This delay is due to the time taken for equilibration in the gas exchanger and for the blood to reach the foetus The length of the tubing on the venous side *the inclusion or not of a dialyser in this* part of the circuit and th flow rate all make a difference to it

Fig — hows the effects of a ga change from 20% O_2, 5% CO_2 and N_2 to 5 O_2, 5 % CO and N_2 and back again. The O saturations, the heart rate and the flow rate are given. The foetus w about the



Fig. 3.

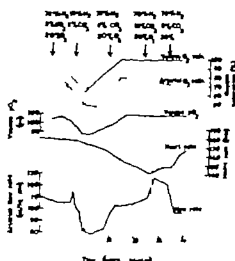


Fig. 4.

same age as the one which gave the previous record but the effect of reducing the concentration of O_2 in the gas mixture was different. The heart rate fell and with it the flow rate and both recovered in the after period. This is the effect on the flow rate which has nearly always been obtained.

Fig. 3 shows another record of a change from air to N_2 and in this experiment a return to air. The O_2 saturation, the pO_2 in the umbilical artery and the flow rate are given. This time the change to N_2 reduced the flow rate almost to nothing and the heart rate probably fell but the flow rate improved afterwards. The timing of the lowest point on each of the 4 records is instructive. The arterial pO_2 was the last to improve.

Fig. 4 shows the effects of changing the gas mixture in a younger foetus from 20 % O_2, 5 % CO_2 and N_2 to 5 % CO_2 and N_2 only and then through the first mixture again to 20 % O_2, 10 % CO_2 and N_2 and finally back to 20 % O_2, 5 % CO_2 and N_2. The first change produced a questionable rise of flow rate at first followed by a fall, without any change in heart rate. During the recovery period the heart rate fell slowly while the flow returned to its original rate. The change from 5 to 10 % CO_2 in the gas mixture raised the flow rate without materially affecting the heart rate.

There are several comments to be made on these records.

1) It was hoped that in an isolated system such as this one reproduceable results would be obtained but this has clearly not always been so any more than it has been so with the placental circulation intact [1, 17]. Hypoxia produced inconsistent effects on the flow rate and it is not yet

possible to say why According to Dawes [7] both the degree of hypoxia and the stage of gestation must be taken into account but in spite of this it is difficult to integrate our experiences with those of Cox [6] and there is evidently more work to be done in this field to clear up the matter

2) In these experiments the heart rate has been no guide to the flow rate. It may remain the same while the flow rate alters considerably as in Fig 1 and 4 or it may vary with the flow rate and appear to initiate the change (Fig 2)

3) Greenfield, Shepard & Whelan [11] found the flow rate in a non viable human foetus of 98 days gestation to be 45 ml/kg min. Dawes [7] reported that the flow rates found by himself and his colleagues in the intact mature sheep foetus were of the order of 132–260 ml/kg min. The flow rates in our isolated pig foetuses may be too low and Callaghan and his associates [3] are worried bout theirs for the same reason. Ours have varied with this placenta from 20 to 110 ml/kg min and may not yet be physiological. They are certainly very variable

It was difficult to make satisfactory long term metabolic observations while the gas tensions were being varied at short intervals, but placenta "A" is quite suitable for such work and Fig 5 is record of the pH, lactic eid, glucose and fructose in the circulating blood of a 61 day foetus taken over a period of 3½ hours. No changes were made to the foetus or the circulation once it had been satisfactorily established. It is hard to maintain foetus as young as this in physiological stat for long and after about one hour the pH of the circulating blood began to

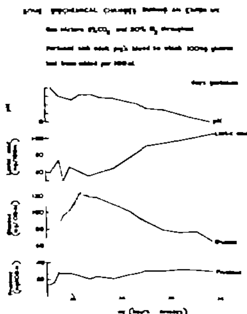


Fig. 5.

fall steadily and the concentration of lactic acid to rise. There was no dialyser in the circuit to correct this. The level of glucose in sow's blood has usually been found to be about 50 mg/100 ml and it was customary to raise this level by 100 mg/ 100 ml t the beginning of an experiment and again later if this was thought necessary In the experiment shown in Fig 5 after the initial rise of glucose, the concentration of this sugar fell steadily and this has been assumed to be due to its utilisation. The rise in fructose shown in Fig 5 has frequently been observed and has been interpreted in the light of the experiments of Huggett [13] as being due to diffusion out of the foetal tissues, but more work on this subject is being planned.

Fig 6 shows the way in which a foetus deteriorates towards the end of an experiment. After a steady period of * to ½

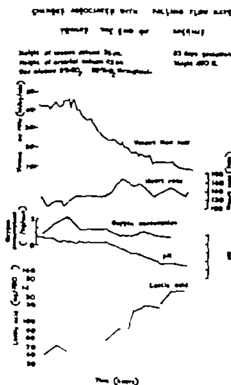


Fig. 6.

hours the flow rate fell over the next 4 hours from about 40 ml/kg min to about 10 ml/kg min while the heart rate remained about the same (120–130 beats/min), and the oxygen consumption was reasonably well maintained. Nevertheless the pH of the blood began to drift downwards and the lactic acid rose. The concentration of other metabolic acids may also have increased [18] but this was not investigated.

Fig. 7 shows a record made on a Grass Recorder of the effect of spontaneous foetal movements on the arterial and venous pressures. The movements were mostly flexures of the trunk and limbs and each was followed by a rise in venous pressure and immediately afterwards a small spike in the arterial pressure.

Placenta B'

When a pig foetus is first cannulated the flow rates in the umbilical vessels may be 100–?00 ml/kg min and the blood pressure 20–30 mm Hg. These are much higher flow rates in the umbilical vessels than have ever been achieved with placenta A. Within half to three quarters of an hour however, the blood pressure may have fallen to a low level. At this stage the foetus is highly sensitive to the volume of blood on the venous side of the heart. The removal of a small sample of blood for analysis may lead to a sudden fall of blood pressure as registered on the arterial manometers and the sudden death of the foetus.

THE EFFECT OF FOETAL MOVEMENTS ON BLOOD PRESSURE IN THE UMBILIC L CIRCULATION

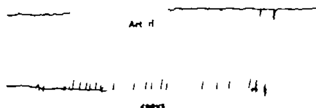


Fig. 7



Fig 8.

The addition of a small volume of plasma to the venous side may re-establish a vigorous circulation after what had appeared to be imminent failure Fig 8 illustrates this As the venous and arterial pressures fell from their initial levels of 28 and 13 mm Hg over the first half hour the flow rate also fell. At the first vertical line 5 ml f plasma was added to the venous side of the circulation. There was an immediate rise of arterial and venous pressures and of the flow rate and th heart rate fell When the arterial pressure had fallen lowly again 5 ml of saline were added to the circulation with a similar but more ephemeral effect In another experiment with a pig foetus of 64 days gest tion and approximately 100 g weight the arterial blood pressure 10 minutes fter catheterization w *0 mm Hg and the umbilical flow rate very high at 187 ml/kg min. During the next five minutes the blood pressure and the flow rate began to fall. At this point instead of adding plasma the blood pressure was raised to the original level of *0 mm Hg by adjusting the screw clamp on the polythene tube leading from the umbilical arteries to the gas exchanger There was a slight initial rise in flow rat but after about _ minutes the flow rate fell to about 100 ml/kg min. During th next 50 minutes th flow rate and blood pressure remained steady though there was a slight rise in blood pressure to 22 mm Hg A gradual fall in both blood pressure and flow rate then set in so that at the end of another 50 minutes the blood pressure was 15 mm Hg and the flow 81 ml/kg min. It may therefore be necessary not only to keep up the filling pressure from the venous side but also to maintain a reasonably high blood pressure in the external circuit by mechanical means, and we shall have to investigate this

Discussion

The progressive engorgement of the foetuses in placenta \" is probably one aspect of the oedema and the engorgement which is well known to be the fat of all perfused organs. The present set-up shows that it is not due to the absence of cardiac pulsation or movements as has been suggested and one must think of some other cause The difficulty in establishing a permanent circulation in placenta B is certainly an example of the delicate adjustments now known to be necessary if it is proposed to maintain physiological pressures flow rates and blood volumes in any ystem involving an extra-corporeal circulation [5 8 9]

present studying these problems. The difficulties should not be insurmountable if it is proposed to use a placenta of either type for short periods of time only as a resuscitation measure but difficulties will have to be overcome before foetuses can be maintained in a physiological state for long periods of time

Since the two placentae give such different results a comparison of the pressures and flow rates given by them in the extra-corporeal circulation has now been initiated and is yielding valuable information. The effects of varying the umbilical arterial and venous pressures and of raising and lowering the amniotic pressures are being studied first and these will be reported in a short time.

Summary

1) Some of the results obtained with pig foetuses surviving in the artificial placenta, previously described are shown.

2) The shortcomings of the original instrument, its evolution and the incorporation of the recording gear are illustrated by the records.

3) A new type of instrument is described which involves different principles, is more physiological and gives rather different results.

4) Comparisons are drawn between the two instruments and this work is being continued

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Changes in Plasma Fibrinolytic Activity of Newborn Infants during First Hour after Birth

by LARS ENGSTRÖM and LARS KAGER

The occurrence of fibrinolytic activity in plasma from umbilical cord blood has been established by several authors [2, 3 6 7] The fibrinolytic activity is not transferred from mother to infant but arises independently in the fetus or placenta The initiating factors are unknown

In a previous study [8] it was shown that in most healthy fullterm infants a significant fibrinolytic activity is found in the plasma within one hour after birth Blood from vena porta or inferior vena cava was analysed using the method described by Blombäck [5] It was even observed that the fibrinolytic activity was more pronounced if the in vitro incubation was performed at a lower pH (6.3) than at a higher pH (7 1) Also in a similar investigation (in patients suffering from liver cirrhosis) increased fibrinolytic activity at a lower pH was observed explained by the authors as possibly due to an enhanced activation of plasminogen to plasmin at a lower pH than at a higher one [4]

The differences in fibrinolytic activity between the single individuals in our previously presented investigation [8] may be caused by blood sampling at different points of time during the first hour after birth Berglund [3] has shown that the fibrinolytic activity of plasma disappears completely during the first three days after birth and it is possible that the diminution is considerable already within the first hour

In order to evaluate how fast the diminution in fibrinolytic activity is during the first hour after birth following investigation was performed

Material and Methods

Eleven healthy fullterm infants were examined. The iliac artery was catheterized through one of the umbilical cord arteries and sample of blood was drawn within the first minut after delivery The catheter was left in the same position and two more blood samples were taken, one after 10 minutes and one after 60 minutes. The fibrinolytic activity was determined according to Blombäck [5]. The plasma was incubated at pH 6.3 and double tests were performed. A reduction of fibrin of at least 300 μg during one hour incubation was calculated as a significant fibrinolytic activity [8].

Results

The results are presented in Table 1

In all cases there was a significant lytic activity either in sample 1 or in sample 2. However in only two cases a significant lytic activity was detected in sample 3 In both these cases the activity was significantly lower than in sample 2.

TABLE 1. *Fibrinolytic activity in plasma of iliac artery at 1 minute, 10 minutes and 60 minutes after birth.*

Case No.	Fibrinolytic activity (µg/hr) 1 minute	10 minutes	60 minutes
1	274	1 03[a]	310
2	800	0	—
3	1300[a]	1330[a]	400
4	873	1220	0
5	330	315	0
6	0	870	0
	800	1635[a]	0
8	1433[a]	1820[a]	0
9	640	1870[a]	0
10	1600[a]	1000[a]	—
11	1060[a]	1130[a]	—

Complete lysis.
[b] Uncertain value due to hemolysis. Not significant lytic activity.

Discussion

Fibrinolytic activity in plasma seems to be a normal finding in healthy infants at delivery. The activity however decreases rapidly and in most cases it is not possible to detect any activity in the arterial blood one hour after birth.

This change of the activity may depend on the circulatory and respiratory adaptation to extrauterine life which takes place after delivery. Before onset of respiration the blood in the iliac and umbilical arteries is venous while the blood in the umbilical vein represents the most arterialised blood of the infant. When a normal pulmonary gas exchange is established the oxygen saturation of the blood in the iliac artery increases. By means of repeated analyses during the first hour after birth the following changes were found in O and CO — tension in iliac artery and in vena porta (9). In the iliac artery P_{O_2} rose within a few minutes from an initial value of 10–20 mm Hg to about 40–60 mm Hg and then continued to rise [illegible] slowly. P_{CO_2} increased rapidly during the first minutes from about 40 mm Hg to 60–80 mm Hg but then it decreased to a level of 40 mm Hg in about 30 minutes.

If the fibrinolytic activity in plasma from the iliac artery in [illegible] infants is compared with the previously reported activity in vena cava [illegible] at approximately one hour of age the fibrinolytic activity seems to be more pronounced in the venous [illegible] any definite conclusions [illegible] rence in fibrinolytic activity [illegible] terial and venous blood [illegible] since a simultaneous [illegible] performed. Some support [illegible] sis may be given by [illegible] tion (3). According to [illegible] vena jugularis [illegible] about 30 [illegible] of the cases [illegible] 4 hours after birth [illegible] using another method [illegible] fibrinolytic activity [illegible]

The pH of the [illegible] in converting [illegible] (fibrinolysin) [illegible] increases at a lower pH [illegible] status of the [illegible] zed by a [illegible] bolic acidosis [illegible] infant [illegible] more [illegible] Gordon [illegible] and [illegible] same [illegible]

This [illegible] tion of [illegible] the [illegible] chan[illegible] [illegible]

Summary

Eleven healthy newborn infants were investigated concerning the degree of plasma fibrinolytic activity in the iliac artery during the first hour after birth. Samples were drawn within the first minute after birth after ten minutes and after one hour The activity was estimated according to Blombäck

Significant fibrinolytic activity was established in all cases during the first ten minutes after birth. After one hour there was virtually no fibrinolytic activity remaining

A possible relationship between the fibrinolytic activity and the changes in blood gases and acid base balance after birth is discussed.

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Fibrinolytic Activity in Plasma of Newborn Infants

by LARS ENGSTRÖM and LARS KAGER

F brinolytic activity—that is the abi lity to dissolve fibrin—is a property of the plasma which occurs when the proen zyme plasminogen is activated and con verted into plasmin. F brinolysis is obser ved in many conditions such as long ope rations, exercise injections f adrenaline etc [1 2, 7 13].

During normal pregnancy and delivery no fibrinolytic activity is found in the ma ternal blood. On the other hand in cases of premature separation of the placenta amniotic fluid embolus or intrauterine death (if the fetus has been dead for bout six weeks before delivery) fibrinolysis may involve maternal risks Fibrinolysis in these cases is secondary to intravascu lar blood coagulation caused by free thromboplastin (thrombokinase) in the blood. Both placenta and amniotic fluid contain large amounts f thromboplastin.

There have been several investigations of fibrinolytic activity in umbilical cord blood of healthy normally delivered in fants. Beller [4] found that in *0–30 % of infants fibrinolysis occurs in the cord blood whilst t the same time no fibrino lytic activity is present in the mother s blood. Ciulla *et al* [10 11] found 96 % of infant to have some fibrinolyti activity in the cord blood and stated that the ac tivity was more pronounced in premature and stillborn infants Chaplin [9] compared the fibrinolytic activity in umbilical artery and umbilical vein blood, and found fibrinolysis in 90 % of the arterial blood samples and in 24 % of the venous blood samples

Berglund [5] investigated the umbilical cord blood in 143 cases using the fibrin plate method described by Astrup & Mül lertz [3] She found a weak or moderate fibrinolytic activity in half of the cases. The activity disappeared during the first three days after birth. No correlation was found between the fibrinolytic activity of the mother s blood and that of the cord. Thus it appears that the fibrinolytic principle is not transferred from mother to hild, but arises independently in the fetus in the placenta.

The factors provoking fibrinolytic activity in the cord blood are not yet known. In earlier investigations as a rule the umbilical cord blood was taken after clamping f the cord, giving a mixture of rterial and venous blood after cessation of the placental circulation. The im of the present investigation was to study the fibrinolytic activity in the circulating blood of the infant shortly after birth.

Material and Methods

The material consists f 25 healthy full term infants. Within one hour after delivery

was made on 25 healthy fullterm infants within one hour after birth. Significant fibrinolytic activity was observed in most cases. The lytic activity increased as the pH decreased from 7.1 to 6.3. Plasma fibrinogen concentration was not influenced by the amount of fibrinolysis. Fibrinogenolytic activity could not be established in any case.

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Cytological Aspiration Biopsy in Adolescent Goitre

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Adolescent goitre is generally regarded as a special kind of goitre appearing in prepuberal and puberal age The typical adolescent goitre is small, soft and diffuse The goitre is regarded by many as due to compensatory hyperplasia of the thyroid elicited by endocrine changes during puberty [13 27], but the underlying goitrogenous factors are unknown. It is, however known that goitre in adolescence as in other periods of life may be a manifestation of various thyroid disorders. Thus, in Boston [21] and Philadelphia [14] lymphocytic thyroiditis is common among adolescent girls with goitre and iodine deficiency was the probable cause in a Danish series of juvenile atoxic goitre [15]. Environmental and hereditary factors are probably responsible for differences in type and incidence of thyroid dysfunction at puberty

Needle biopsy of the thyroid gland to obtain tissue for histological examination is useful in the differential diagnosis of thyroid disease in adults [2, 6 7 11 23 26] and in children [5 14 21]. A simpler method introduced in Sweden by Söderstrom [24] is the thin needle biopsy with cytological examination of the aspirated material [4 9 17 24]. This technique was used in the differential diagnosis of adolescent goitre in the present investigation.

Material

Thyroid disease was diagnosed in altogether 80 children living in Gothenburg and seen at Gothenburg Children Hospital (the only children hospital in the city) from September 1959 to February 1963. Gothenburg is a seaport with somewhat more than 400,000 inhabitants and situated on the west coast of Sweden, where nothing suggests dietary iodine deficiency The present investigation was limited to children aged 10 to 16 years with non-toxic goitre. This category consisted of 60 girls and 3 boys. Of these 8 had no palpable thyroid gland at the follow-up for needle biopsy one had undergone operation for thyroid adenoma and one for thyroid cyst In 5 biopsy was refrained from mainly for psychological reasons. In the remaining 48 patients needle biopsies were performed. In the beginning of this study both large (for histological examination) and small bore biopsy needles were used, but later only small ones because the aspiration of material for cytological study is much simpler In 9 cases both large and small needles were used; in 39 a thin needle only

In most cases the goitre was asymptomatic and detected at routine medical examination. The girl operated upon for thyroid cyst was the only sister of a girl with cystic thyroid, which had been punctured with a thin needle

Methods

Biopsy specimens of the thyroid were obtained with a small-bore needle in the way described by Söderström [23]. The needle

has an outer diameter of 0.7 mm. It is fitted to the syringe with a Luer lock so that it may be manipulated with one hand [10]. Local analgesia was used in only a few cases. Specimens were obtained from both thyroid lobes, often from several areas. The needle was repeatedly moved to and fro during continuous aspiration. The aspirated material was ejected onto a glass slide and several short thin smears were prepared without delay The smears were air-dried stained by the May-Grunwald-Giemsa method and examined by one (S P) of us.

Thick needle biopsy specimens were obtained with a corkscrew type of instrument [3].—These biopsies were performed by Dr P Heimann and the pathological examination of the specimens was done by Dr L. B Schnürer

The sera were studied (Dr Deborah Doniach, London) for thyroid antibodies. The methods and results will be reported in a separate paper [8]; here the serological findings will only be studied for any correlation with the cytological diagnoses.

Results

The cytological diagnoses are given in Table 1 In 4 patients biopsy was repeated in order to get representative material, but even then the material obtained in one case was not sufficient to warrant a firm cytological diagnosis though the cells found suggested thyroiditis. The procedure was tolerated well and was not attended by bleeding or other complications.

1 *The cytological picture of the normal thyroid gland*

Smears of aspirated material from normal-sized thyroid glands of healthy adults contain thyroid follicular epithelial cells, small amounts of thyroid colloid and a varying quantity of peripheral blood (unpubl observations) The follicular epithelial cells are of fairly uniform appearance. They have round nuclei with rather loose chromatin structure Cellular outline is usually not distinct. The cytoplasm has a bluish tint and contains vacuoles and paravacuolar granulae (Fig. 1) A few lymphocytes from the peripheral blood are seen.

2 *The cytological picture of chronic lymphocytic thyroiditis*

The cytological abnormalities in our young patients with thyroiditis were the same as those known to occur in adults with Hashimoto's disease A detailed report of the cytological findings in this disease will be published elsewhere [10], here only the main features will be mentioned.

All smears except one were rich in tissue cells of mainly two types lymphocytes-reticular cells and follicular epithelium (Fig 2) The diagnosis was based on the presence of lymphocytes and reticular cells. The lymphocytes were predominant. Reticulo-endothelial cells with signs of marked phagocytic activity (histiocytes, macrophages) were common. Plasma cells were occasionally seen (Fig 3) In fact in many cases the picture closely resembled that of chronic non-specific lymphadenitis.

There were no specific changes of the follicular epithelium cells, which were often of normal appearance A varying degree of pleomorphism was observed in some cases with a wide range of variation in size shape and staining properties of the nuclei (Fig 4) This pleomorphism was often accompanied by a reduction or disappearance of the paravacuolar granulae. Debris material was engulfed in some cells,

and in a few the cytoplasm had undergone foamy vacuolization. Only 3 smears contained appreciable amounts of colloid. No fibrous or vascular elements were observed. Most smears contained only a small admixture of blood.

3. The cytological picture of colloid goitre

All cases in which the smears contained colloid and thyroid follicular epithelium cells but no accumulation of lymphocytes or reticular cells were assigned to this group The colloid was often the most striking feature As a rule the follicular epithelial cells looked normal, but in some preparations the cell nuclei varied in size and staining properties and contained large nucleoli (Fig 5) These proliferative changes suggested an increased cell activity Foamy phagocytes were encountered in _ cases only

4 The cytological picture of cystic goitre

Thin cyst fluid was aspirated in _ cases. In one of them the gross appearance of the fluid suggested an old haemorrhage in the cyst Microscopically foamy phagocytes represented the dominant type of cell (Fig 6) The follicular epithelium cells were scanty and poor in detail.

Cytological and histological diagnoses

Nine specimens were studied cytologically and histologically The cytological findings were invariably typical of chronic lymphocytic thyroiditis. The histological features were consistent with the diagnosis of chronic lymphocytic thyroiditis in 4 cases In one case the very small specimen consisted mainly of ordinary thyroid follicles with colloid, but contained some inflammatory cells in the margin, where the tissue was too severely damaged to allow evaluation. The remaining 4 specimens contained no demonstrable thyroid tissue

Cytology and serology

The cytological evaluation and the serological studies were performed separately by independent examiners. A good correlation was found between the cytological diagnoses and the occurrence of thyroid antibodies (Table 2). The clinical and serological findings in the patients with juvenile lymphocytic thyroiditis will be reported in detail in separate papers [8 18].

Cytology and type of goitre

The type of thyroid disorder could not be recognized with certainty by palpation of the goitre In only bout half of the patients with cytological evidence of thyroiditis had this diagnosis been suggested by the palpatory findings. In most patients with a small soft and diffuse goitre the cytological diagnosis revealed colloid goitre

Discussion

Historical data and clinical findings are rarely sufficient to establish a differential diagnosis in non-toxic goitre in children. In some cases radioiodine studies may yield useful information. Demonstration of thyroid antibodies suggests an autoimmune disease of the thyroid. But the presence of thyroid antibodies in a low titre is not conclusive evidence of diffuse lymphocyti thyroiditis, at least not in adults, for thyroid antibodies may ppear also in patients with other kinds of thyroid disease in patients with diffuse collagen disease without overt thyroid disease and even in healthy subjects [1 1

TABLE 1 *Cytological diagnoses based on thin needle aspiration biopsies in 48 juvenile patients with non-toxic goitre*

	Both sexes	Girls	Boys
Chronic lymphocytic thyroiditis	28	25	3
Prob. chron. lymph. thyroiditis	1	1	—
Colloid goitre	19	19	—
Cystic goitre			—
Total	48	45	3

20 with further ref., 22]. Absence of thyroid antibodies as judged by the tanned red cell agglutination and complement fixation tests does not rule out thyroiditis [18]. It would therefore appear that thyroid biopsy is often indicated in the investigation of thyroid disease.

Thyroid tissue for histological examination can be obtained by open surgery or with a large bore puncture needle, while a small needle is sufficient for aspirating material for smears to be studied cytologically. The histological methods have certain advantages over the cytological procedure: they provide information on the general appearance of the lesion, the degree of involvement of different elements and the anatomical relations between different types of cells and fibrous and vascular structures. As to the cytological procedure, it allows examination of the structure of the individual cells and the proportions between different types of cells, preparation of the material is simple and the cells can be studied under the microscope after a short interval. Patients are more willing to consent to small needle biopsy in the examination room than to large needle biopsy in the operating theatre.

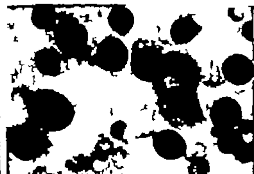
The cytological method seems to give reliable results. Thus, in their study of thyroid cancer Einhorn & Franzén found no false positive cases, but in [illegible]% of the whole series the method failed to reveal signs of malignancy seen at subsequent operation. In the present series all cases with a significant titre of thyroid antibodies were diagnosed as chronic lymphocytic thyroiditis by cytological aspiration biopsy (Table 2). The patients with colloid goitre had no demonstrable thyroid antibodies apart from 1 with antibodies against the second colloid antigen, the significance of which is uncertain. The number of patients examined with both the histological and the cytological methods was too small to warrant any comparison, but in a large material of adults with Hashimoto's disease the histological and cytological findings were in almost complete agreement [19].

The cytological picture of chronic lymphocytic thyroiditis was largely characteristic and allowed a positive diagnosis without hesitation. Negative smears do not, however, exclude the possibility of lymphocytic thyroiditis. The cytological picture was characterized by usually abundant lymphoid cells and changes, though sometimes slight, in the morphology of the epithelial cells. The findings thus resembled the histological characteristics of juvenile lymphocytic thyroiditis [21, 28]. However, it may sometimes be difficult to distinguish lymphocytic thyroiditis from thyrotoxicosis from the cytological appearance alone, but this problem is beyond the scope of the present investigation. Goitres with lymphocytic thyroiditis and colloid goitres differed considerably in colloid content of the smears. Thus, colloid


Fig. 1


Fig. 2


Fig 3


Fig 4

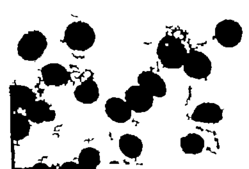

Fig 5


Fig 6

E PLA TI PLATE

Fig 1 The normal thyroid gland Follicular epithelial cell with para acinolar granulae Cytoplasmic borders not identifiable (40

Fig Chronic lymphocytic thyroiditis low power survey A group of follicular epithelial cell in the centre surrounded by lymphocytes and reticular cells (100).

Fig 3 Chronic lymphocytic thyroiditis One re ulo-endo helial cell h phagocyte ne ma terial the left, one plasma cell surrounded by lymphocytes t the righ of he picture (4)

Fig 4 Chronic lymphocytic thyroiditis Polymorphism of follicular epithelial cell 40

Fig 5 Colloid goitre Proliferating follicular epithelial cell and thin colloid (40

Fig 6 Cystic goitre Foam phagocytes (40)

TABLE ... *Cytological diagnosis and thyroid antibodies*

Two patients with thyroiditis and one with colloid goitre were not studied for antibodies.

	N of pat	Microsomal antigen: CFT pos.	Microsomal antigen: CFT neg. cyto pos.	Thyroglobulin TRC-titre: 1/25,000	Thyroglobulin TRC-titre: 5–250	2nd colloid antigen (CA2) pos.	N thyroid antibodies
Chronic lymphoc. thyroiditis	24	15	5	1	8	4	0
Prob. chron. lymphoc. thyroiditis	1	0	1	0	1	1	0
Colloid goitre	18	0	0	0	0	2 (weak +)	16
Cystic goitre	2	0	0	0	0	1	1
Total	45	15	6	1	9	28	17

CFT = complement-fixation test.
TRC = tanned red cell agglutination.

was demonstrated in the smears of only 3 of the patients with thyroiditis but in all of the smears from patients without cytological and serological evidence of thyroiditis. The cytological appearance of the colloid goitres was sometimes indistinguishable from that of the normal thyroid gland, but the colloid content was often larger and epithelial cell changes were sometimes seen

The high incidence of lymphocytic thyroiditis in the present largely random selection of adolescent goitres and the reports from Boston [21] and Philadelphia [14] show that the condition is much more common in children than widely supposed. Before the present study had been started no cases of thyroiditis had been diagnosed at this hospital which suggests that "the increasing occurrence of thyroiditis" [16] is not true but only apparent and due to the increased attention now being given to the disease

Summary

Thin needle biopsies with cytological examination was used in the differential diagnosis of non toxic adolescent goitre. During a period of three and a half years 63 children (10–16 years) with non toxic goitre were studied. Biopsies were performed in 48 of them. Chronic lymphocytic thyroiditis was found in 27 cases, colloid goitre in 19 and cystic goitre in 2 cases. The procedure was well tolerated by the patients and no complications were observed. Almost complete agreement was found between cytological diagnosis and presence of thyroid antibodies. The cytological characteristics of the chronic lymphocytic thyroiditis: the abundance of lymphoid and reticular cells, was easily recognized and allowed a firm diagnosis. Chronic lymphocytic thyroiditi i apparently more common among adolescent with goitre than widely supposed

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Late and Early Operations for Cryptorchism

by C. G. BERGSTRAND and O. QVIST

During previous follow up studies [3] of boys treated for cryptorchism the im pression was gained that orchiopexy per formed at an early age i.e. during the first few years of life, gave a less satisfactory final result with regard to the position and size of the testis than prepuberal operations. As the problem of the best time of operation is still open to debate it was thought of interest to study a series of boys operated upon at an early age in comparison with a series treated during the prepuberal years. If early orchiopexy could be demonstrated to give less satisfactory results, this would be an argument in favour of post poning the operation.

Materials and M thods

Three series f patients were studied. The first comprised all boys who had undergone orchiopexy before the age f six years during the period 1933 through 1937 In all, 52 cases had been operated upon and with a few exceptions all had demonstrated an inguinal hernia, which had been the immediat cause f operation. None had been treated with hormones.

For comparison we used second group f patient who had been operated upon during the same period but at the age f 9 t 14 years and who had had a hernia diagnosed before the operation. The total number f patient in this group was 41 The age distribution t operation in both groups is given in Fig. 1

Of the 52 patients of the first group, 51 were re-examined and the corresponding figure for the second group was 40. The re examination was made 1 to 9 years fter the operation. In one case in the younger age group and one in the lder information was obtained from the parents, as an exa mination was not possible and in a few cases the examination was carried out in other hospitals. In both groups re-operations were performed on 7 and 5 patient respectively and in these the result fter the second operation was considered as the final result. In both groups there were cases with bilateral cryptorchism (10 and 4 respectively) and as in these patients the operation was not always performed on both sides, the results will be presented with regard to the number of testes operated on and re-examined rather than with regard t the patients treated. In the first group 58 testes were operated on and re-examined and in the older group 43. At the follow-up examination the size consistency and position f the testis was evaluated and the result f the operation was considered as good when these criteria were found t be normal.

A third group f patients was also in cluded in the material. This group consisted f 102 boys operated upon during the same period before the age f 6 for an inguinal hernia without cryptorchism. These patients were selected at random from the case files for each of the years 1933 to 1937 The number for each year was roughly the same and the age distribution corresponded with that of the first group. T the parents of these patients was sent a questionnaire When the information obtained was not satisfactory i.e the parents were uncertain



Fig. 1. Age distribution at the time of operation in groups 1 and 2.

about the result or considered it as unsuccessful the patients were called to the hospital and re-examined. For different reasons no information was available about 6 patients. Of the other 96 patients 77 were re-examined in person.

The operation methods were the same throughout the relevant period.

Results

The primary result of the orchiopexy according to the available case notes was satisfactory in group 1 and 2 in about 78 % of the operations. It was stated that the testis had been of normal size and had been placed in the scrotum. In the younger age group the operation had been unsuccessful in 12 cases out of 58 one patient had a testicular aplasia on the operated side and in one patient the case notes did not give any details of the result. In the older age group 9 operations gave an unsatisfactory result and in two patients the testis was found to be smaller than normal.

Both groups were found to be comparable with regard to the testicular position before the operation. Between 60 and 70 % of the testes were situated in the neighbourhood of the external ring a smaller number was found in the canal and in both groups only a few had an abdominal position.

At the follow-up examination 41 testes of the 58 operated in the younger age group were found to be normal with regard to size consistency and position. The corresponding figure for the older group was 27 out of 43. Expressed in percentages, the result was satisfactory in 77 and 63 % respectively. In both groups the rest of the testes showed an abnormal position and/or a more or less pronounced atrophy. In the younger age group one testis was already missing at the operation, an extrascrotal position with or without demonstrable atrophy was found at the follow up in 8 cases and 10 testes were atrophic with or without an abnormal position. In all 12 testes (23 %) were in some way or other abnormal in this group. In the older age group 16 testes (37 %) were atrophic but two of these had been found smaller than normal already at the operation. In this group no testis was found in an abnormal position without being smaller than normal.

In the third group of 96 re-examined patients who had been operated upon for

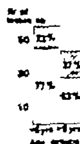


Fig. . Comparison of late results in groups 1 and 2. Unsatisfactory results ■ Good results □ Number of testes operated in younger age group: 58. Number of testes operated in older age group: 43.

an inguinal hernia before the age of 6 years, 1 patient with testicular abnormality was found. This patient had a definite testicular atrophy on the operated side

Discussion

The results of the present investigation do not substantiate the original impression that orchiopexy performed in infancy or early childhood gives a less satisfactory final result than operations in the prepuberal years. On the contrary the figures rather point in the opposite direction. Hallman *et al.* [7] found the same tendency in their follow up study In the older age group of the present investigation unsatisfactory results were recorded at the follow-up examination in 37 % of the operated testes compared with 23 % in the younger group. The difference is not very large but significant. There are however other factors to be taken into consideration when the groups are compared. There is little doubt that the two groups of patients with undescended testes are comparable with regard to the pre-operative position of the testis and the existence of an inguinal hernia. The immediate operative results were also the same in both groups and are in fair agreement with the results of large series of operated undescended testes [5, 6]. When two different age groups are compared, as in the present investigation, it must be kept in mind that an older group in a sense may be an unfavourably selected one Patients whose testes eventually would have undergone spontaneous descent are necessarily included in a younger age group and may be more favourable objects of operation. It is well known and has recently been confirmed by Rundle & Sylvester [8] that the testes increase little during childhood. The more pronounced and rapid growth occurs at puberty This implies, and it is emphasized by Hallman *et al.*, that a testis congenitally defective or damaged at an early operation may be found definitely hypoplastic or atrophic only after the patient has reached puberty For this reason it is necessary to draw the conclusions of the present results very cautiously In the younger age group only a small minority of the patients had reached puberty whereas most of the older patients were adolescents at the time of the follow up. To get a quite comparable figure the younger age group ought to be followed at least to puberty and the present investigation does not warrant the conclusion that prepuberal operations give less satisfactory final results. Even if, on the other hand, it cannot be wholly excluded it seems very unlikely that a re-examination of the younger age group during or after puberty would so change the figures that it could be shown that early orchiopexy gives a less satisfactory result than prepuberal operations.

The third group of patients who had been operated upon for an inguinal hernia not combined with undescended testis was selected to serve as control group if the early operations (first group) yielded a high incidence of primary failures. It is known that surgical repair of inguinal herniae in childhood is in a certain number of cases followed by testicular atrophy According to Andreassen & Lindenberg [1] this incidence is very high when the operations are performed in departments

of general surgery and not by paediatric surgeons. This statement is not confirmed by a recent investigation by Fahlström *et al* [4], who found an incidence of testicular atrophy of 1 % following hernial repair in young boys. Bauer & Hesse [2] give the incidence of testicular atrophy after hernia operations as 3 1 %, but this figure apparently concerns adults. In the present investigation about 1 % of testicular abnormalities were found. The figure is, for obvious reasons, not entirely reliable. In two-thirds of the material the evaluation of the results was based only on observations by the parents. Even if such information according to Hallman *et al* is fairly accurate it seems wiser not to draw too extensive conclusions. It must also be kept in mind that most of these patients had not reached puberty. In spite of the uncertainty of the results, it seems unlikely that the figure for testicular damage following the surgical repair of inguinal hernia can be very much *higher in the present material than the 1 %* given.

Summary

A group of 52 children, who had been operated upon before the age of 6 years for an inguinal hernia combined with undescended testis, was re-examined with regard to their testicular status. It was possible to get reliable information on all except one. This group was compared with a group of 41 patients who had undergone surgery for the same combination in the prepuberal years. In the latter group 40 patients were re-examined. The primary operative results were the same in both groups. The final results were slightly better in the younger age group, but it is pointed out that most of the patients in this group had not reached puberty. Testicular damage may not be apparent before this time and for this reason the two groups were not entirely comparable.

A third group of 102 infants and children of the same age as the first group operated upon for an inguinal hernia, was also followed up and it is concluded that the *incidence of postoperative testicular damage* was probably about 1 %.

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Free Fatty Acid and Glucose in the Blood of Various Groups of Newborns Preliminary Report

by V MELICHAR, M. NOVÁK, P HAHN and O KOLDOVSKY

Immediately after birth, changes in the RQ indicate a preferential utilization of lipids [1 6]. Van Duyne & Havel [2] and Novák *et al.* [4] showed that immediately after birth the blood-glucose level falls, while the free fatty acid (FFA) content of the blood increases. The intravenous administration of glucose prevents this rise in FFA content [4] It has been also shown [3] that the relationship between FFA and glucose contents in the blood is different in the newborn than later in life

In the present paper this relationship has been studied in more detail.

The blood levels of FFA and glucose were determined about 12 hours after birth in several *groups of newborn infants* (Table 1) Fig. 1 shows that in full-term normal infants there is a negative correlation between these two indicators. In hypotrophic infants (born at term but

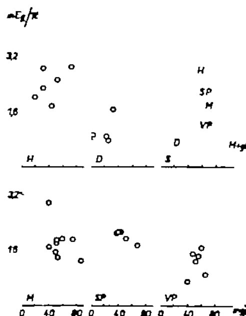


Fig. 1 Relationship between blood glucose (abscissa) and free fatty acid (ordinate) content in newborn infants. *M* = *full term infants*, 0.672, p 0.05, *SP* = slightly premature infants, – 0.95, $p < 0.02$, *VP* very premature infants, *H* hypotrophic infants, *D* infants from diabetic mothers, *S* summary Infants given glucose load are also shown = *M* gl (20 ml of 10 solution into the umbilical vein and 80–110 ml of 10 solution (average 103 ml) by mouth in first 9 postnatal hours—6 healthy newborn babies). Statistical significance of differences in summary: ( ) In blood-glucose levels in different groups D lower than all other groups, $p < 0.01$ M gl higher than all other groups, p 0.001 (b) In FFA levels M SP $p < 0.05$, M VP $p < 0.02$; M H, $p < 0.002$; M D p 0.005; VP H $p < 0.001$ VP SP $p < 0.001$ SP H, not signif.

TABLE 1 *Distribution of newborn infants*

	Body weight (g)	Gestational age (weeks)
Slightly premature	1800–2400	34–37
Very premature	1240–1750	28–32
Hypotrophic	1350–2310	36–41
Diabetic mother	3120–4580	33–37
Full term	2850–4200	38–41

weighing much less) a low level of glucose and a high level of FFA were found. In newborn infants of diabetic mothers both levels were very low. Premature infants always had low levels of glucose [5, 6, 7], those born between weeks 28 and 32 showing very low levels of FFA, while those born between weeks 33–36 had high FFA levels.

These differences indicate either differences in the amount of stored fat or a different utilization of carbohydrates and fats. The second possibility is indicated by the fact that in full term normal infants and slightly premature infants there is a significant correlation between glucose and FFA content (as is usual for adults), while in other groups this is not the case. It is hoped that further data may help us to define "hypotrophic" infants more exactly.

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Respiratory Studies in Children. XII

Serial Studies of Mechanics of Breathing Lung Volumes and Ventilatory Capacity in Provoked Asthmatic Attacks[1]

by INGA ENGSTRÖM

Among the diagnostic procedures to which asthmatic patients have to submit the provocation test is of major importance The aim of the test is to assess whether a suspected allergen may elicit asthmatic symptoms on inhalation. In children in whom specific and polyvalent allergies are common [13] this test is repeatedly performed in the same patient to distinguish between latent and manifest allergies [3 7 1_]

Inhalation of allergen to which the patient is sensitive leads to airway obstruction with increased pulmonary flow resistance [8 15] decreased flow of air as assessed by measurements of forced expiration [1 14 16] and uneven ventilation a assessed by measurements of nitrogen wash-out during oxygen breathing [4].

However our knowledge of the duration and degree of airway obstruction during provoked attacks is limited. Nor is it known whether the airway obstruction adds to the hyperinflation often met in asthmatic children.

This study provides further information concerning the immediate effect of a provoked asthmatic attack upon the mechanics of breathing lung volumes and ventilatory capacity as well as concerning the duration of this effect. The results are also compared with those obtained in spontaneous attacks.

Material

Nine asthmatic children between the ages of 6 and 14 were studied, 5 boys and 4 girls. The children were selected among those attending the Allergy Out Patient Clinic t the Pediatric Department f Karolinska Sjukhuset Stockholm.

The clinical characteristics of the children are summarized in Table 1 The duration f disease varied from months to 12 years. In three children the duration was less than one year The frequency of asthmatic ttacks during the year prior to study assessed in the same way as in a previous study of spontaneous ttacks [11], varied between 1 and 16 The severity f the asthmatic attack was judged subjectively as in the previous study with group 1 as the slightest and group 5 as the most severe [11]. The majority of the children had slight and only two children ery severe ttacks. N hild was or had ever been on corticosteroid therapy

[1] Aided by grant from the Swedish National Association against Heart and Chest Diseases.

Table 1 *Clinical data on children studied*

	Age	Height cm	Duration of disease years	Number of attacks last year	Intensity of studied attack	Allergen
B. A.	6.9	128	6	5	5	Horse epithelium 1/10 000
G. B.	12.5	156.5	0.3	3	1	Horse epithelium 1/10 000
E. L.	9.5	142	8	10	1	Thimothy pollen 1/100
P. M.	8.0	124.5	2	1	1	Birch pollen 1/10 000
G. O.	12.8	143	3	16	5	Horse epithelium 1/1000
L. S.	10.8	139.5	0.5	10		Cat epithelium 1/100
G. S.	14.3	149	9	14	1	Mould 1/10
R. T.	11.8	154	0.5	3		Alder pollen 1/100
P. W.	14.4	150	13	12	2	Thimothy pollen 1/10

Methods and Procedure

The children in this study were investigated during the same period as the children with spontaneous asthmatic attacks previously reported [11]. The same methods were applied. Pulmonary flow resistance (R) and dynamic compliance (C) were obtained by simultaneous recording of intraesophageal pressure changes and respiratory volume changes via a "reverse plethysmograph" system previously described [9, 11]. The static lung volumes were measured by spirometry and the closed circuit helium dilution technique, and the timed vital capacity by spirometry with a fast moving kymograph.

All children were first examined at symptom-free status with measurements of lung volumes, forced vital capacity and mechanics of breathing consecutively. In 7 children this examination was performed immediately before provocation, and in two children 5 days before provocation. During the time interval the children were in a stable symptom-free status without symptomatic therapy. The attacks were provoked by inhalation of nebulized allergen solutions to which the patients previous history and skin tests indicated that they were sensitive. The inhalations were performed with a Pari Optimal inhalator which nebulizes 1 ml allergen solution in around 5 minutes, the majority of the droplets being 0.5–5 μ. The symptoms started within 5–30 minutes after provocation and the recording of the pulmonary function took place immediately after the symptoms became manifest. The mechanics of breathing were determined first and after that the lung volumes and the forced vital capacity. As the symptoms gradually diminished during the test they were much less pronounced during recording of lung volumes and forced vital capacity than during recording of mechanics of breathing. In some children no dyspnoea or rhonchi remained at the end of examination. This change in clinical status was reflected in a significant difference between duplicate determinations of the static lung volumes. The mean difference in V_{FRC} was -0.230 l and in V_{VC} 0.246 l. In view of these differences the mean of duplicate determinations was not used in the calculations of V_{FRC}, V_R, V_{VC}, V_{TLC} and the ratios

TABLE 2 *Pulmonary function data*

Case		Day of study	V_{FRC} l	V_R l	V_{VC} l	V_{TLC} l	FEV l	V_{VC} forced l	C ml cm H_2O	R cm H_2O/l/sec
B. A.	symptom-free	−1	0.90	0.46	2.04	2.50	1.36	1.82	45	18.3
	attack	1	1.40	1.20	1.11	2.31	—	—	24	55.0
			1.19	0.93	1.54	2.47	0.72	1.28	—	—
	symptom-free	2	0.83	0.50	1.92	2.42	1.48	1.93	65	15.9
	symptom-free	4	0.80	0.46	2.02	2.40	1.43	1.94	—	—
G. B.	symptom-free	−1	1.81	0.90	2.78	3.67	2.45	2.90	70	5.9
	attack	1	1.81	1.43	2.45	3.78	—	—	24	37.5
			1.37	0.86	2.70	3.56	2.20	2.69	—	—
	symptom-free	2	1.43	0.68	3.04	3. 2	2.68	3.03	70	3.9
	symptom free	6	1.47	0.90	3.10	3.79	2.48	2.99	78	4.6
E. L.	symptom free	1	0.96	0.32	2.26	2.68	1.75	2.19	41	8.8
	attack	1	1.36	0.66	1.94	2.62	—	—	34	20.6
			1.09	0.47	2.14	2.62	1.67	2.08	—	—
	symptom free	2	1.03	0.38	2.22	2.60	1.96	2.17	30	8.0
	symptom-free	3	1.03	0.39	2.20	2.65	1.75	2.13	44	7.3
	symptom-free	4	1.06	0.46	2.23	2.70	1.86	2.15	37	8.5
P. M.	symptom-free	1	0.90	0.41	1.86	2.27	1.67	1.85	35	6.8
	attack	1	1.01	0.74	1.20	1.94	—	—	15	44.2
			1.00	0.73	1.49	2.22	1.06	1.63	—	—
	ronchi	2	0.83	0.60	1.31	1.91	0.71	1.15	26	20.4
	symptom-free	3	0.76	0.42	1.81	2.03	1.05	1.44	32	9.3
	symptom free	4	0.80	0.39	1.74	2.13	1.30	1.67	43	6.6
G. O.	symptom-free	1	1.62	0.94	2.43	3.37	1.88	2.50	78	9.1
	attack	1	1.90	1.63	0.56	2.11	—	—	24	97.0
			—	—	—	—	0.29	0.49	—	—
	symptom-free	2	1.40	0.96	2.31	3.29	1.40	2.22	55	14.2
	symptom-free	3	1.84	1.21	2.57	3.78	1.96	2.65	69	10.2
	symptom-free	4	1.66	1.02	2.67	3.09	1.87	2.54	—	—
L. S.	symptom-free	1	1.34	0.67	2.62	3.19	2.03	2.66	65	3.6
	attack	1	1.50	0.91	2.32	3.23	—	—	47	13.1
			1.39	0.63	2.62	3.25	1.20	2.03	—	—
	symptom-free	2	1.42	0.61	2.77	3.38	2.16	2.63	77	8.4
	symptom-free	3	1.46	0.60	2.82	3.41	2.17	2.63	70	4.1
G. S.	symptom-free	1	2.13	1.28	3.04	4.32	2.01	2.93	139	7.2
	ttack	1	2.30	1.78	2.10	3.85	—	—	62	16.7
			2.08	1.44	2.37	3.81	1.31	2.40	—	—
	symptom-free	2	2.10	1.19	2.90	4.09	1.68	2.84	109	4.8
	symptom free	3	2.17	1.19	3.16	4.35	1.99	3.11	137	5.3
R. T.	symptom-free	1	1.89	0.90	2.95	3.86	1.99	2.90	124	8.4
	attack	1	2.12	1.41	2.57	3.98	—	—	45	33.5
			1.81	1.00	2.96	3.95	1.00	2.29	—	—
	ronchi	2	2.09	1.23	2.67	3.87	1.26	2.57	73	9.8
	symptom-free	3	1.72	0.77	3.04	3.81	1.67	2.92	95	5.4
	symptom-free	4	1.78	0.72	3.09	3.80	1.86	3.08	110	5.5
P. W.	symptom-free	1	1.88	0.78	2.63	3.44	2.17	2.69	120	3.0
	attack	1	2.03	1.84	0.83	_.72	—	—	6	22.6
			1.65	1.43	.91	2.44	0.67	1.10	—	—
	symptom-free	2	1.58	0.85	2.48	3.33	1.90	2.49	65	3.8
	symptom free	3	1.61	0.75	2.60	3.44	2.26	_.65	126	4.6

TABLE 4. *Pulmonary function dimensions during attack in per cent of individual symptom free pre-attac kvalues*

	Mean	SE	SD	Range
V_{FRC}	1 17	5.–	15.5	108–156
V_R	167 6	13.3	39 9	137–201
V_{TLC}	91.2	4.5	14.0	63–106
$FEV_{1.0}$	55 6	8.4	–5.1	18–83
V_{VC} forced	70.1	8 0	4.0	20–100
C	46.6	6.6	19.8	22–83
R	540.8	94.1	283.5	406–1060

as slight (degree of severity 1 and 2) Only two children had severe attacks (degree of severity 5) and none had attacks labeled as 3 or 4 The distribution of children according to intensity of attack was thus very uneven, and no close relationships between the pulmonary function aberrations and this factor were found.

The lack of relationship between bronchial obstruction, as reflected in the increase in R, and the disability of forced ventilation, as reflected in the decrease of $FEV_{1.0}$ and $FEV_{.75}$ may be due to the differences in clinical status. R measured in the beginning of attack when the symptoms were most marked might not be representative of the bronchial obstruction one hour later when $FEV_{1.0}$ was measured and the symptoms were lesser or nonexistent The same factor may be responsible for the poor relationship between bronchial obstruction and hyper inflation as manifested in a probably significant relationship between R and the ratios but none between R and V_{FRC}.

The day chosen for cross-sectional analysis at symptom free status after attack was the one with the lowest pulmonary flow resistance determined This day was obtained early one to three days after attack and all pulmonary function values were by then almost identical to pre-attack values.

Longitudinal study

For evaluation of the change induced by attack the values were analysed as a percentage of symptom free pre-attack values. Table 4 gives the means with SD SE and range for the different pulmonary function measurements. Fig 2 illustrates the individual change in pulmonary function during and after attack.

The marked increase in R during attack returned to pre-attack level one to two days after attack. Only in one child (P M.) was R still markedly increased on the day after attack and did not return to pre-attack level until 3 days after attack.

C decreased at attack and returned to pre-attack level one to three days after attack.

Forced vital capacity and $FEV_{1.0}$ decreased at attack, the latter regularly more than the former The return to pre-attack levels however followed the same pattern and was similar to that for C which is visible from the figure The child (P M.) with a high R of longer standing than the other children had a lower forced V_{VC} and $FEV_{1.0}$ on the second day of observation than immediately after provocation, and the values were still low 3 days after attack An observation one week later showed values close to pre-attack level.

V_{FRC} was increased at the first determination during attack, but had decreased at the second determination to values closer to those before provocation (91 132% of pre-attack value) On the day

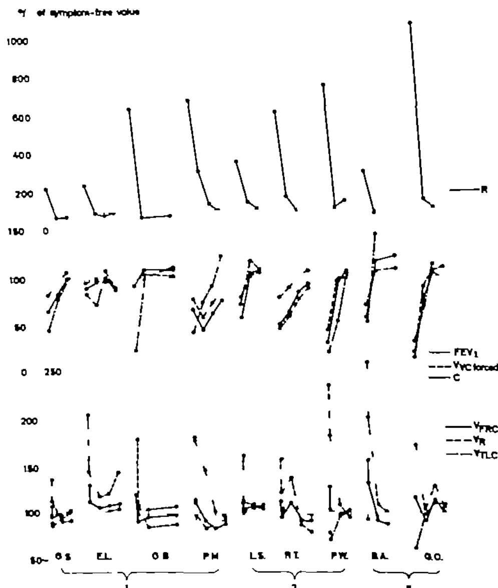


Fig. _ Pulmonary function measurement during and after provoked asthmatic attacks, indicated as percentage of individual pre-attack values. Numbers 1, _, and 3, indicate degree of severity of attack as judged clinically

after ttack V_{FRC} was in all children again close to pre-attack level (mean 99)

V was also markedly increased t the first observation during attack and had decreased at the second observation 20 minutes later The values at this moment however were still rather high (109–20* ,.) The pre-attack level was reached one to two days after attack except in one child (E. L). (The pre-atta k

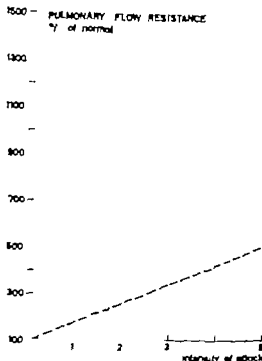


Fig. 3. Pulmonary flow resistance at attack in relation t intensity f attack as finically judged. —— regression line for the same relationship in spontaneous attacks.

V_R was only 58 per cent of predicted normal. Hence in per cent of predicted normal the value was normal already by the second day.)

V_{TLC} was fairly unchanged during and after provocation in 5 children. In 4 children V_{TLC} *decreased moderately* at attack but increased to pre-attack level within the same period as the other lung volumes.

Comments

It is evident that all pulmonary parameters change at the provoked attack and that *a rapid return to pre-attack level* in general takes place after attack. Occasionally however the pulmonary function may remain abnormal for several days after provocation *even if only minor* symptoms such as rhonchi remain one day after provocation (P. M. R. T.).

There is a tendency to discrepancy in normalization rate between R and $FEV_{1.0}$, *with a time lag for the latter.* The most rapid return occurs in V_{PAO}, which in some cases is normal already within the first hour after provocation.

The rate of normalization in the different pulmonary function parameters does not seem to be correlated to the degree of deviation at attack.

Comparison between pulmonary function in provoked and spontaneous attacks

The change in pulmonary function in provoked attack is similar to that in spontaneous attacks [11], though the degree of change in each particular dimension may differ between the two forms of attack. An estimate of the probable difference must take the intensity of attack into consideration.

When R in provoked attacks is plotted against intensity of attack (Fig. 3), all values but one are above the regression line for the same relationship in spontaneous attacks. This suggests a difference between the two materials. Plotting of the other pulmonary function dimensions into corresponding diagrams of spontaneous attacks does not disclose a difference like the one in R. The majority of values mingle with those in spontaneous attacks. This holds true also for $FEV_{1.0}$, which was registered when the symptoms had declined and in some *patients totally disappeared.*

The probable difference in R between the two materials was tested by comparing the mean values in per cent of symptom free values in the 7 children with slight

TABLE 5. *Mean of individual pulmonary flow resistance and functional residual capacity n per cent of symptom free values in provoked attacks severity group 1 and 2 compared to correspond g mean values in spontaneous attacks, severity groups 1–3 and 4–5 respectively*

		Mean	Difference	Significance of differences
R	Spontaneous attack 1 3	257 1	13.9	$P < 0.01$
	Provoked attack 1 2	301.0	–4	$P > 0.05$
	Spontaneous attack 4–5	496.6		
V_{FRC}	Spontaneous attack 1 3	11–6	5.0	$P > 0.05$
	Provoked ttack 1 2	117.6	16.3	$P < 0.02$
	Spontaneous ttack 4–5	133.9		

symptoms (degree of severity 1 and 2)[1] in the provoked attacks with the cor responding values in the 10 children with slight to moderate symptoms (degree of severity 1,– and 3)[2] in the spontaneous ttacks and in the 11 children with severe attacks (degree of severity 4 and 5) separately [11]. The results are shown in Table 5

R was significantly higher in the slight provoked attacks than in the slight to moderate spontaneous ttacks the in crease being as high as in the severe spontaneous attacks.

As a correlation was found in the spontaneous attacks between the mcrease in V_{FRC} and R [11], V_{FRC} was compared in the two materials in the same way as R (Table 5)

There was no difference between the mean increases in V_{FRC} in the lght provoked attacks and in the slight to moderate spontaneous ttacks but there was a significantly lower V_{FRC} in the provoked attacks than in the spontaneous ttacks with severe symptoms

Comments

The analysis of the difference in increase of R and V_{FRC} in provoked and spontaneous attacks thus shows that the provocation of an asthmatic attack tends to elicit a more marked bronchial obstruc tion than does spontaneous asthmatic attacks, however without a comparable increase in V_{FRC}. Thus there is a discrepancy between bronchial obstruction and hyperinflation in the provoked attacks com pared to spontaneous attacks which sug gests that the pulmonary reaction in provoked attacks is not identical to the one in spontaneous asthmatic attacks, lso when disregarding the rapidly changing status during a provoked attack. However by simultaneous registration of airway resistance and lung volume by means of body plethysmograph technique [2, 6] it would be possible to make a more relevant evaluation of probable differences in pulmonary reaction.

Discussion

The provocation test is expected to give reliable information about bronchial al lergy without simultaneous harmful effect upon the pulmonary function. To avoid or minimize the hazards of impairing the

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[1] Four children belonged to severity group 1 and 3 to severity group 2.

[2] One child belonged t severit group 1 3 to severity group –, and 6 to severity group 3.

pulmonary function by provocation, only minimal symptoms should be elicited by using allergens in appropriate dilutions.

As previously found, signs of bronchial obstruction can be detected by an increase in pulmonary flow resistance when symptoms are minimal or even in the absence of symptoms [8]. The provocations in this study were performed with the aim of eliciting only minor symptoms. This was achieved in seven children. In two however the allergen solutions chosen provoked severe asthmatic symptoms.

A marked bronchial obstruction was induced in all children. Pulmonary flow resistance increased even more than is usually observed in spontaneous asthmatic attacks although the latter regularly show more clinical signs and symptoms [11]. The attacks, even when accompanied by severe symptoms, were of short duration clinically. Most of the signs and symptoms had disappeared within an hour to an hour and a half. On this account all pulmonary function dimensions were not measured at the same clinical status. The changing clinical status is reflected in particular in the measurements of the static lung volumes. The first determination, performed immediately after the measurement of R and C, showed high functional residual capacity. Twenty minutes later this volume was close to normal again. Thus a hyperinflation is induced in a provoked attack, but of very short duration.

It is probable that the obstruction of the airways diminishes at the same time as the lung volumes normalize and the symptoms decline. However still one to one and a half hours after provocation, there are signs of impaired ventilatory ability with a low $FEV_{1.0}$ and a low forced V_{VC}. Thus, even if the bronchial obstruction is not prominent enough to maintain a hyperinflation during quiet breathing obstruction of the airways may exist forced breathing.

It seems that a pulmonary reaction induced by allergen inhalation can be easily recognized by increase in R and decrease in $FEV_{1.0}$ even if only minor symptoms are present. The bronchial obstruction during quiet breathing as reflected in a high R is usually of short duration but may occasionally remain a few days, even if the immediate symptoms are mild. A hampered ability to forced ventilation with decrease in $FEV_{1.0}$ and forced V_{VC} may remain one or several days after the induced attack even if no symptoms are present.

The marked increase in R with only a moderate increase in V_{FRC} immediately on provocation of mild asthmatic symptoms is contrasting to the findings in spontaneous attacks [11], in which an equivalent increase in R is consistent with a clinically very severe attack and often accompanied by a more marked increase in V_{FRC}. The short duration of hyperinflation is also in contrast to the findings in spontaneous attacks in which hyperinflation may remain also when R has returned to normal.

It seems probable that the shortstanding bronchial obstruction in a provoked attack does not influence the ultimate end expiratory level and thus does not add to the hyperinflation often met in asthmatic children. However the possibility can not be excluded that repeated induction of severe attacks may affect the end expiratory level.

Summary

Asthmatic symptoms were provoked by allergen inhalations in 9 children with bronchial asthma.

Mechanics of breathing lung volumes and ventilatory capacity were registered before during and after provocation. Pulmonary flow resistance functional residual capacity and residual volume increased in all children, and vital capacity forced expiratory volume in 1 sec. decreased. Total lung capacity was uninfluenced or decreased.

Pulmonary flow resistance increased to a higher value than is usually reached in spontaneous attacks of the same clinical degree of severity The deviations of the other pulmonary function dimensions were similar to those in spontaneous attacks. The increase of pulmonary flow resistance usually was of short duration. The decrease of FEV $_{1.0}$ sometimes remained one or several days after attack The hyperinflation induced was of very short duration.

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Late Manifesting Variant of Branched Chain Ketoaciduria (Maple Syrup Urine Disease)

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In 1954 Menkes, Hurst & Craig [7] described a new syndrome, later shown to be due to an inborn error in the metabolism of leucine isoleucine and valine, which were excreted in pathological amounts in the urine [6] This branched-chain amino-aciduria was associated with a peculiar odor of maple syrup of the urine The disease is therefore commonly called maple syrup urine disease The first report comprised 4 cases out of 6 siblings, and later reports have added 11 cases distributed in 7 families [12] Symptoms usually developed within the first week of life with seizures intermittent rigidity and loss of Moro's reflex as early manifestations. The disease was in most cases rapidly fatal. In those living longer mental deficiency was the most prominent symptom except in two cases treated with a diet low in branched-chain amino acids [10 11]

Assuming that only gross metabolic errors would manifest itself in the neonatal period, one might expect the existence of a variant of the disease being due to a partial enzyme deficiency or other minor metabolic error This variant might either give rise to less distinct symptoms or manifest itself only under special provocative conditions. Little is known about the symptomatology and occurrence of this variant since only a single case previously has been described [8] This was a girl who since the age of 16 months had recurrent periods of ataxia and semicoma, when the urine had the typical odor and contained large amounts of leucine isoleucine and valine. Morris *et al.* [8] believed that this was an apparently nonfatal variant.

In the present report two additional cases with a slightly different symptomatology are described. It is shown that this variant has a familial occurrence that it may manifest itself for the first time as late as in the 8th year of life as an acute, fatally ending disease.

Case Reports

Case 1

U F a girl born June 1961 and the second child of unrelated and healthy parents, both 35 years old and without any previous history of illness. The child was born at term after a normal pregnancy Weight at birth 3900 g, length 52 cm Because of cyanosis and respiratory distress, presumably caused by pothidine given to the mother during delivery she was admitted to the Pediatric Department where she recovered within a few hours. Since the ge

of 6 months she several times developed otitis media bilaterally Spontaneous per foration usually occurred after a few hours During these ttacks, often associated with high fever she never had any neurologic symptoms or signs. Her next stay in the Pediatric Department was in Sept 1962 Three days prior to admission she developed bilateral otitis media with fever (39°C) and spontaneous perforation. The next day she was afebrile but suddenly developed attacks of 3–4 minutes duration when the body became stiff in opisthotonus. In the course of the day she had more than twenty attacks and became more and more lethargic.

On admission she was 15 months old, well-nourished, well-developed with fair skin, blue eyes and very blond hair She was afebrile but unconscious, only responsive to painful stimuli. There was purulent secretion from the right ear through a dull thickened tympanic membrane with small perfora tion. Electroencephalogram showed slow activity without any focal signs. The cerebrospinal fluid contained no cells and the con centration of total protein (13 mg/100 ml) was normal. She had metabolic acidosis with total-CO in serum 7 mEq/L. Chloride levels were normal. There was a slight hypopotassemia with 2.9 mEq/L as the lowest recorded value. Blood sugar urea, sediment tion rat leucocyt count hemoglobin, sodium, cal cium and phosphorus determinations showed normal values. The urine contained protein. Rothera and Gerhardts reactions were posi tive In the days after admission a strong, peculiar odor was noticed and described as reminding of curry Chromatogram of the urinary amino acids showed increased excre tion of leucine and valine There was also present a spot in the hromatogram believed t be due t methionine? She remained unconscious for three days and had d ring this time several attacks of tonic and lonic convulsions with torsion spasms of the extremities.

She was treated with luminal nd chloral for her convulsions. She recei ed intravenous infusions of saline glucose and sodium lactat which resulted only in a slight increase in total CO_2. She was no longer comatose after three days and the peculiar odor of the urine disappeared. The first 10 days in the hospital she received no antibiotics, but was later treated with sulfonamides and penicillin on account of pyuria with growth of E. coli in the urine. Two days after discharge she was readmitted with symptoms of otitis media bilaterally (39°C). The course was uncomplicated and the urine was without any peculiar odor

She is now 28 months ld, physically well developed and of normal weight and length. Her ocabulary is larger than usual for her ge and psychological xamination using the Terman Merill and Gesell tests suggest that the hild is normally developed. Since her stay in hospital the parents have three times noticed a slight odor of the urine when she had normal temperature. At two oc casions she had infections with temperat re to 40°C without any peculiar smell of the urine Chromatography of the urine at several occasions have showed no pathological findings. The mother has been told t restrict the intake of milk and to withdraw milk during periods f fever A synthetic diet low in branched-chain amino acids [9] is in store if the hild should go into new period f illness.

Case 2

B. F., born March 1955, the 6-year-older brother of the preceding case was born t term after an uncomplicated pregnancy birth weight 4060 g H was admitted to the Pediatric Department at 8 months of age on account of vomiting, diarrhoea and fever At admittance the temperature was 38.6 C both tympanic membranes were injected and paracentesis was performed on both ears with the emptying of a small amount of pus containing Staph. aureus from the right ear The cerebrospinal fluid contained a normal number of cells whereas the total protein concentration was definitely in creased (99 mg/100 ml). E cept vomiting there were no other signs or symptoms suggesting involvement of the central ner ous system. EEG findings were normal





Fig 1 Fig 2.

Fig. 1 Chromatogram of amino acids in the serum of the 8-year-old boy (Case 2) 1 leucine-isoleucine 2: valine

Fig 2. Chromatogram of amino acids in the serum from healthy boy

Before discharge two weeks later second puncture revealed normal cerebrospinal fluid with total protein 19 mg/100 ml

H was admitted January 1963 to the Epidemic Department under suspicion of meningitis. With the exception of few periods in wintertime of acute bronchitis, last one more than a year ago h had n healthy and developed normally physically and mentally By his schoolteacher h was considered one of the brightest boys in the class. Three days bef re admission h complained of pain in the head and the stomach, and the temperature rose t 39°C In the course of the following days he vomited repeatedly and had no intake of food. He became increasingly lethargic

On admission he was a nearly 8 year-old well-developed boy with blue eyes, f ir skin and very blond hair He was f brile with frequent respiration. The pupils were dilated, but reacted to light He was semi comatose with high pitched cries. There were frequent torsion spasms of the extremities. EEG examination showed slow ctivity over all regions without any focal signs. The cerebrospinal fluid contained 11/3 cells totalprotein 48 mg/100 ml and sugar 48 mg/100 ml. There was no growth in culture and viral studies were negative He had metabolic acidosis with total CO m serum 9 mEq/L, chloride 100 mEq/L, sodium 140 mEq/L potassium 5.1 mEq/L and calcium 5 6 mEq/L. Sedimentation rate 6 mm/1 hour hemoglobin 14 1 g/100 ml, leucocyt count 6900/mm with normal distribution, blood-sugar 84 mg/100 ml, urea 47 mg/100 ml. Th urine was negative for protein, sugar and blood but strongly positive for acetone (Rothera's reaction). There was a strong curry-like smell of the urine reminding of the odor of the urine of his siste when she was comatose 4 months previously

Serum amino acids. A blood sample was drawn on the day of admission. The serum wa deproteinized by mixing serum-ethanol (1–4) An aliquot corresponding t 60 μl serum was hromatographed on thin layer (Silica gel G Merck) using CHCl -CH OH 17% NH_3 (2 1 w/w) and Phenol H_2O (75–25, w/v) as running solvents. Fig 1 shows the amino acid chromatogram which is characterized by excessive amount of





Fig 3. Fig. 4.

Fig 3. Case 2. Chromatogram of amino acids in the urine from Case 2. 1 leucine-isoleucine; 2: valine; 3: histidine; 4 cystine

Fig. 4 Chromatogram of amino acids in the urine from Case 1 in normal period

valine and leucine-isoleucine The last two amino acids were not separated with the system used Fig 2 shows a typical serum amino acid hromatogram from healthy boy

Amino acids i the urine. An amount f urine containing 20 μg of creatinine was chromatographed on silica gel thin layer Fig 3 gives the amino acid chromatogram. This shows a massive excretion of leucine soleucine (not separated) V line excretion is also considerably increased The excretion of histidine is low whereas the excretion of cystine is relat vely high. Fig 4 shows a normal amino acid chromatogram of urine *recently btained from the sister of the pati* ent (Case 1)

Keto acids the urine Ket acids were determined the same urine sample as that one used for amino acid chromatography but fter 9 months store in the deep freeze Using the method f McArdle [4] modified by Dent & Westall [2] the 2,4-dinitrophenyl h drazones of the keto acids were xtracted and hromatographed on silica-gel thin layer using as running solvent butanol-ethanol 0.5 N NH OH (7 1 /v) Fig 5 how the chromatogram of 2,4-dinitrophenylhydra zones from the urine of the boy For com parison the corresponding chromatograms from two healthy boys are also presented. The chromatogram from the urine of the patient shows two ery strong spots which had the same R_f as the tw hydrazoneisomers of ketoisocaproic acid (the keto acid of leucine). The thin layer with the two spots was scraped off eluted in 3 ml of a mixture of 10% N CO and 5 N N OH (1-1) measured at 440 mμ in spectrophotometer and recorded as -ketoisocaproic acid. The urine contained 143 mg ketoisocaproic acid/g creatinine Because of overlapping *on the chromatogram between two f the* hydrazone-isomers of -ketoisocaproic acid and pyruvic acid, the total extinction of the hydrazones in the R_f area of these t o substances was also measured and corresponded t 156 mg/g creatinine Control values in 10 healthy boy ranged from 6 6 t 32.5 mg/g creatinine whereas the amount of ketoglut aric acid/g creatinine amount w at the lower end of the normal range possibly on account of long-time storage I order t get more certain identification, the mixed hydra



Fig 5. Chromatograms of the 2,4-dinitrophenyl hydrazones of the ketoacids in urine from 2 healthy boys (*A* and *B*) and from Case 2 (*C*).

zones from —5 ml of urine were dissolved in 2 ml of glaciale acetic acid, 3–4 drops of 2 N HCl were added and the hydrazones electrolytically hydrogenated to the corresponding amino acids in a desalting pparatus [5]. The chromatogram of these aminoacids showed large amounts of leucine-*iso*leucine whereas valine was not found. There was less glutamic acid (from α ketoglutaric acid) and less alanine (from pyruvic acid) than usually found in normal urine The findings indicat the presence of either α ketoisocaproic acid (from leucine) or/and α keto-β methylvaleric acid (from *iso*leucine)

In summary the serum concentration of the branched amino acids leucine and/or *iso*leucine and valine was greatly raised when the boy was comatose and these aminoacids were excreted in xcessive amounts in the urine The ketoacids correspond ing to leucine/*iso*leucine were also found in high concentration in the urine

Cl nical course He was treated with infusions of glucose and sodium lactat but lapsed shortly after the admission into coma. He had frequent convulsions lasting 10 minutes or more and responded poorly to anticonvulsive treatment It became increasingly difficult to keep his airways free He was intubated and put on artificial respiration using an AGA respirator after a considerable period of respiration stop when he became deeply cyanotic. Later a tracheotomy was performed In addition to respi ratory failure there was also a cardiovascular failure with dropping blood pressure and he had the last two days of his lif to be kept on Aramine which was added to the in fusion fl ids. He died 5 days fter admission.

Autopsy No significant findings were made in any organ except the brain, which was edematous and weighed 1780 g No gross focal lesions were observed in the grey or white matter Microscopical examination showed a considerable spongy degeneration of the deep layers of the cortex (Fig. 6) whereas the nerve cells were fairly ell preserved. The hippocampus showed no lesions. The most impressive changes ere found in the cerebellum. Here the molecular and Purkinje-cell layers were well preserved while there was a pan necrosis of the entire

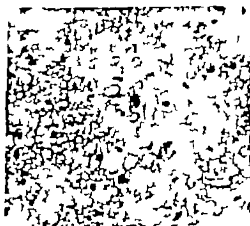


Fig 6 Spongy degeneration in the deep layers of the cerebral cortex (Case 2).


Fig. 7


Fig. 8.

Fig. 7 Section from cerebellum showing necrosis of granule cells and preservation of other layers. Weil' stain (Case 2).

Fig. 8. Detail of preceding picture showing preserved molecular and Purkinje cell layers and necrotic granule cells.

granule cell layer (Fig 7 and 8). There was also considerable nerve cell loss in the pontine nuclei and in substantia nigra. There were no glial or vascular hanges. The white matter of the cerebrum and cerebellum was normal.

Discussion

Most cases of maple syrup urine disease previously reported developed their symptom within the first months of life The symptoms of the two children described in this report appeared for the first time when they were 15 months and nearly 8 years old. Together with the 16-month-old girl described by Morris *et al.* [8] these cases evidently represent a late mani festing variant In none of these cases ha there been a progressive neurologic deterioration which is so characteristic when the disease starts in the neonatal period. The peculiar urinary odor has only been present for short periods, usually when clinical symptoms developed. The acute ttacks have much in common with those of the neonatal type the main symptoms being ataxia lethargy respira tory distress and coma associated with high blood levels and increased urinary excretion of leucine isoleucine and valine and the ketoacids of these branched chain amino acids.

Normally the branched-chain amino acids are transaminated to the corre sponding α-ketoacids. Through oxidative decarboxylation the ketoacids are con verted into the next lower fatty cid. Since th branched-chain amino acids and ketoacids accumulate together in maple syrup urine disease it is assumed that the metabolic defect is localized to the oxidative decarboxylation step [13]. In the late manifesting variant reported in this paper only the branched-chain amino acids were determined in the serum The question therefore arises whether the metabolic block of this late manifesting variant is localized to another m tabolic step The excessive metabolic acidosis and the large mounts f ketoacids ex creted strongly suggests that the defect

is not localized to the transamination step. A block further down in the metabolic pathway would lead to the accumulation of fatty acids in the blood. Since the step of oxidative decarboxylation is irreversible it is unlikely that the branched chain amino acids would accumulate if the block was to the right of the keto acids. It is therefore assumed that the metabolic defect has the same localization as in the more common variant with onset in the first months of life. Dancis *et al.* [1] suggested on account of leucocyte studies that the disease is due to a defect in an enzymatic step common to all three branched-chain ketoacids in their degradative pathway. Since the children with the late manifesting variant have lived without symptoms for years and developed physically and mentally in a normal way one might believe that the block is at the same enzymatic step but that the enzyme deficiency is less complete. Another possibility which is not yet ruled out is that the early and late manifesting variants of the disease are due to deficiency of two different enzymes involved in the oxidative decarboxylation.

A partial enzymatic defect might only give rise to symptoms when the amount of substrate is highly increased either when unusual catabolic stress supervenes or when the dietary intake of the branched chain amino acids is high. High endogenous protein break-down is likely to occur during occasional childish ailments, especially when associated with fever and hunger. In the girl the onset of the acute attack had a clear connection to the appearance of otitis. She has however had high fever in connection with otitis media without the manifestation of clinical symptoms or the peculiar odor of the urine. Recently the curry-like odor was noted at two occasions without any association with illness. Chromatography showed only traces of branched-chain amino acids. In connection with the ordinary children diseases the boy had higher fever than at the ailment which started the fatally ending disease. In the girl reported by Morris *et al.* [8] the recurrent episodes of ataxia possibly were associated with periods of teething according to the parents. No apparent correlation was found between the total protein intake and the excretion of keto acids in the random specimens examined.

This girl and the two cases reported here have many features in common. All were fair-haired, fair skinned and blue-eyed children. This syndrome in the same way as phenylketonuria (Follings disease) [3] may therefore be associated with lack of pigment. Important differences in symptomatology however also exist. In the case described by Morris *et al.* [8] episodes of ataxia recurred from the age of 16 months until the case was reported when the child was 41 months old. In contrast coma for three days has been the single episode in the girl. In the boy the fatally ending disease was the first attack. Since this metabolic error may give rise to a fatally ending disease at the age of 8 years maple syrup urine disease should be seriously considered in all cases of acute acidosis, coma, convulsions and fever of unknown etiology throughout childhood. Since the urine odor may be absent even in a fatally ending case [1*], the search for branched-chain ketoaciduria should possibly not be restricted to the cases with a peculiar odor of the urine.

The electrolyte aberration may be of importance in the diagnosis of such cases. The characteristic finding was a pronounced metabolic acidosis with normal sodium and chloride levels. This would suggest the presence of high levels of anions not determined. Having ruled out uremia and more common metabolic disorders such as diabetes mellitus, the normo- or hypo-chloremic acidosis would most likely be due to the presence of organic anions ordinarily not determined in the clinical routine laboratory.

A diagnosis was not made when the children lapsed into coma. One might therefore in retrospect wonder whether such severe attacks might be influenced essentially by a change of treatment. Infusion of glucose and $NaHCO_3$ were in both cases started immediately after the admission but the clinical symptoms progressed. This refractoriness to treatment was probably not due to morphological cerebral damage since the girl was comatose for three days and has completely recovered. A reduction of the blood levels of branched-chain amino acids and ketoacids would probably lead to an improvement of the conditions. Since the molecular weight of these substances are slightly more than one hundred, they probably are highly dialyzable. Treatment with artificial kidney should therefore be considered. Respiratory distress and circulatory failure were the immediate causes of death in spite of all efforts.

On account of the severe respiratory and circulatory dificulties during his last days, the pathologic anatomic picture is difficult to interpret. Obviously cerebral changes due to anoxia might be expected. The cerebral edema and the spongy degeneration of the deep cortical layers might be due to such a cause. However the Purkinje cells and the pyramidal cells of the hippocampus were surprisingly well preserved. On the other hand there was a marked necrosis of the cerebellar granule cells and of several brain stem nuclei. The latter structures are usually preserved even in severe anoxia. Attention should therefore be focussed on these areas of the brain in future cases of the late manifesting type of this disease.

Summary

With 5 months interval two previously healthy siblings, a girl and a boy developed acute metabolic acidosis with cerebral symptoms, coma, tonic and clonic convulsions and respiratory distress. The girl, 15 months old, recovered completely mentally and physically after being comatose for three days. The boy, 8 years old, died on account of respiratory and circulatory failure after 4 days coma. During the comatose periods urine of both patients had a peculiar curry-like odor. Increased concentrations of leucine/isoleucine and valine were present in the serum of the boy. Chromatograms of the urine in both cases revealed increased excretion of these amino acids as well as of the branched-chain ketoacids. The attacks were therefore apparently due to a late manifesting variant of the branched-chain ketoaciduria or maple syrup urine disease. Autopsy of the boy revealed marked necrosis of cerebellar granule cells and of several brain stem nuclei, structures which usually are well preserved even in severe anoxia.

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Familial Sex Linked Thrombocytopenia

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Essential thrombocytopenia may in rare instances occur as familial disease coming on within the first year of life and persisting throughout childhood. The familial form is limited to boys, in contrast to the non familial essential thrombocytopenia which occurs equally often in boys nd girls [20] In a single report on familial essential thrombocytopenia the haemorrhagic diathesis was the only sign [18]. In all other cases the familial thrombocytopenia seems to have formed part of Wiskott–Aldrich s syndrome This syndrome first described in 1936 [1] and "rediscovered in 1954 [1], includes in addition to the haemorrhagic diathesis a tendency to eczema and to infection [2, 3, 4 6 8 11 13 15 22]. Wiskott–Aldrich s syndrome also exists a a non familial disease however and its signs may vary somewhat in intensity In rare cases of familial thrombocytopenia female relatives have also been found to be affected. In two families where persistent thrombocytopenia was diagnosed in boys the mothers of these likewise suffered from the disease but in a very mild degree compared with that in the boys [7 19] In two other families where both boys and girls were affected with thrombocytopenia, the haemorrhagic diathesis did not manifest itself till the age of three years or later [16, 23]. In cases of neonatal thrombocytopenia the mothers may also occasionally have the disease However this neonatal form I neither hereditary nor essential being due to transplacental transmission of platelet agglutinins from the mother [10]. The thrombocytopenia will, in fact subside spontaneously within few months [14].

Below an account will be given of a family with sex linked essential thrombocytopenia, which in a few cases was associated with mild tendencies to eczema and infection. The family relations are illustrated in Fig 1

Case Reports

No. 1 was born in 1924. Throughout childhood he had a pronounced tendency to bru ses and haematomas, but there was no haemorrhagic diathesis after the age of puberty H had no predisposition to eczema or infections, though possibly recurrent otitis media in hildhood. N treatment had been given. Examination in 1963 revealed blood type A, Rh-positive anti B titre 1 128 Direct Coombs test was negative No cow milk precipitins were present in serum. Electrophoretic serum protein fractionation

showed a slightly raised beta fraction (17%) but no other abnormality Leucocyt count and differential count: normal Eosinophils 3%. Clinically well but later he has been admitted to hospital on account of anaemia and thrombocytopenia.

No 2 was born in 1941 Throughout childhood he had a tendency to bruises and epistaxis, and has been subject to abnormal bleeding in relation to dental extractions. There was no tendency to eczema or infections, though he possibly had recurrent otitis media. In 1958 thrombocytopenia was demonstrated. Coombs test was negative leucocyte count and differential count normal no eosinophilia. During steroid therapy the number of pl telets rose transitorily The bone marrow displayed a normal megakaryocyte content. Splenectomy was done in 1959 Microscopic examination of the spleen revealed reticular hyperplasia. After the splenectomy he became symptom free with normal platelet counts. Examination in 1963 disclosed, blood type A Rh positive, anti B titre 1 128. Direct Coombs test negative. No cow milk precipitins in the serum. Electrophoretic serum protein fractions normal. Leucocyte count and differential count showed no abnormality Platelets 166 000 Clinically well

No 3 was born in 1953 The first few years of life he had a tendency to eczema of the face and the posterior surfaces of the knees, as well as to bruises. In 1956 he had pyarthrosis. The platelet count was then 88,000 On examinations during the following years the platelet counts varied from 25 000 to 100,000. In 1960 skin test was found to be positive for egg but an elimination diet and steroid therapy did not improve the thrombocytopenia. No antibodies against platelets were found in the serum. Direct Coombs test was negative. Blood type A, Rh positive (Analyses for iso-agglutinin titre was not performed.) Electrophoretic serum protein fractionation showed a raised alpha$_2$ fraction (19 4%) a slightly reduced beta fraction, and a greatly reduced gamma-fraction (5.3%) In July 1960 splenectomy was done. The plat let count then returned to normal, and the boy became symptom free In June 1961 he died suddenly about 4 hours after a minor injury Necropsy revealed bilateral suprarenal haemorrhage. Reticular hyperplasia and various eosinophils were seen in the lymph nodes. The bone marrow showed pronounced erythropoesis and myelopoiesis and a normal megakaryocyte content but only few plasma cells.

No 4 was born in 1961 He has always had a tendency to bruises and haematomas as well as to epistaxis. In ddition he has shown a predisposition to catarrhal conditions, having had pneumonia four or five times within the first two years of life In 1963 he was admitted to the Dronning Louises Children s Hospital (21/63) During the entire stay he had very frequent bruises, petecchiae and haematomas, as well as twice purulent otitis media, twice pneumonia, and often asthmatic respiration. The skin of the face and limbs was dry red and itching The general condition was good all the time The infections were treated with antibiotics. Laboratory analysis: haemoglobin 11 g/100 ml, platelets 4,000–124,000–60,000. Mild eosinophilia. Thymol, transaminase and prothrombin tests normal. Cow's milk precipitins were not demonstrated Electrophoretic serum protein fractionation repeatedly showed a low gamma content (0 46–0.47 g/100 ml = about 7%) Immunoelectrophoresis showed reduced gamma-globulin, beta 2 A-globulin and beta-2 M globulin fractions and increased beta 1 AC-globulin, alpha-2-macroglobulin and alpha-2 haptoglobulin fractions. Subsequently normal beta-2-A-globulin content and a raised beta 2 M-globulin content were found. Blood type A Rh-positive anti-B titre 1 8. N antibodies against platelets. No LE cells. The diphtheria antitoxin titre was below 0 01 unit despite three previous vaccinations, but after revaccination against diphtheria the titre rose normally The tetanus antitoxin titre and the poliomyelitis antibody titre were normal The bone marrow showed an excessive number of megakaryocytes and an increased number of eosinophils. The faeces

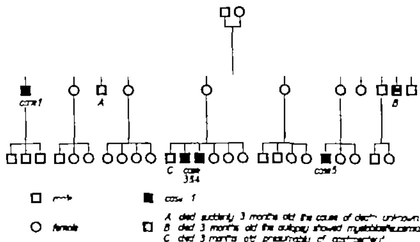


Fig 1.

constantly presented a positive benzidine reaction.

An elder brother had died three months ld after 8 days f vomiting and diarrhoea. \ information is vailable on tendency to infection, haemorrhage or eczema. N post mortem examination was undertaken.

The mother of \ os. 3 and 4 has displayed no tendency to infection, haemorrhage or eczema. Her platelet count was normal on examination in 1962. Iso-agglutinin titre: anti A 1 512 and anti B 1 128 \ anti bodies against platelets. Electrophoretic serum protein fractionation showed slightly raised alpha$_2$-globulin, bet -globulin and gamma-globulin content Immuno-electrophoresis showed raised beta A-globulin and gamma-globulin fractions.

\ o. 4 wa born m 1958 At the age of one year he had eczema of the face and round the genitals. H was treated with hydrocortisone ointment with good response H ha had no eczema since but repeatedly ot tis media. H has always been predisposed t bruises. Examination in 1962 revealed blood type O Rh-positiv ant A titre 1 128, anti A$_1$ titre 1 1*6, and anti B titre 1 16. Platelet 64 000. Platelet agglutinin titre very weakly positive Serum lectrophoresis showed slightly raised alpha fractions. No cow milk precipatins were detected. The diphtheria antitoxin titre was below 0 01 unit despit course of vaccinations four years previously whereas the tetanus antitoxin titre was 0 12 units. The general condition was good all the time

As hild the mother had had a tendency to eczema but not t haemorrhage nor t infections. In 1962 her platelet count was found to be normal. No cow milk precipi tins. Blood type A, Rh-positi e with anti B titre 1 128.

Discussion

In this family as shown in Fig 1 thrombocytopenia occurs solely in boys and seems to be inherited from the mothers. The disease thus presents itself as a recessive sex linked disorder as in the family described by Aldrich *et al.* [1].

The disease manifests itself in early in fancy but the clinical picture varies. The only common feature is that of thrombocytopenia with associated haemorrhagic diathesis. In the first two cases reported above the haemorrhagic diathesis was rather pronounced, but the disease ran

covered spontaneously about the age of puberty while the other became symptom free in response to splenectomy None of them had had eczema or an unquestion able tendency to infections. These two patients presented, in other words, a picture consistent with that described by Schaar [18]. The third patient had both thrombocytopenia and a tendency to eczema, but no history of a tendency to infections. In Nos. 4 and 5 the haemor rhagic diathesis was associated with a tendency to infection and a slight ten dency to eczema. The clinical pictures presented by the three latter patients were thus *consistent with Wiskott–Ald rich s syndrome*, except that the general condition remained good all the time.

The pathogenesis is obscure. The ten dency to eczema and the eosinophilia *suggest an allergic factor* but neither eczema, eosinophilia, nor positive skin tests were constant findings in the family reported above. No decisive proof can be given of an allergic pathogenesis. The same is true of Wiskott–Aldrich s syndrome [4] Patient No 3 had hypogammaglobulinaemia while No 4 had slightly reduced gamma-globulin and transitorily reduced beta-2 A globulin and beta 2 M globulin concentrations in serum suggesting a very mild form of dysgammaglobulinaemia as a possible cause of the tendency to infection. A very low iso-agglutinin titre has been demonstrated in several cases of Wiskott–Aldrich s syndrome [9 11 12, 17]. As the iso-agglutinins constitute part of the beta 2-M fraction, a low titre may be taken to indicate dysgammaglobulinaemia. In our family no remarkably low iso-agglutinin titres were demonstrated. However a normal titre does not militate against dysgammaglobulinaemia or Wiskott–Aldrich s syndrome Thus, in a case of congenital immunoparalysis, where the patient lacked the beta-2-M fraction, fairly great iso-agglutinin titre variations were observed [5] and we have ourselves had occasion to notice a rise of the titre from 1 2 to 1 64 in a typical case of Wiskott–Aldrich s syndrome. The very low diphtheria antitoxin titre in two of our patients who had *previously received* the normal series of vaccinations seemed strange, considering the normal tetanus antitoxin titres of the same two patients. One of these was revaccinated and obtained *a normal rise of the diphtheria* antitoxin titre. The thrombocytopenia seemed to be independent of the infections. The bone marrow was normal in the three cases examined, and no platelet agglutinins were *detected. However a histo*logically normal bone marrow does not preclude a functionally hypoplastic mar row Seip [19] found a normal lifetime of platelets and histologically normal bone marrow in a *patient with familial thrombo*cytopenia. The thrombocytopenia must therefore have been due to a reduced thrombocytopoietic activity of the bone marrow

Splenectomy was performed *in two cases*. One of these patients was then grown up and had never displayed a tendency to infections. He has been symptom-free since the splenectomy The elder brother who presented the same *clinical picture* became symptom-free spontaneously about the age of puberty The other patient became symptom free in response to splenectomy at the age of 7 years but died suddenly one *year later of* suprarenal haemorrhage, without preceding infection.

Of course no conclusion can be drawn from these two cases. It is well known, however, that splenectomy can improve the thrombocytopenia in Wiskott–Aldrich's syndrome [7, 6, 11], but that the patients may nevertheless die of suprarenal haemorrhage [6, 11]. Patient No. 4 has been treated with antibiotics against infection, with a favourable result each time so far. The general condition has hitherto been so good that steroid therapy has been unnecessary. Patient No. 5 had previously had eczema treated with steroid ointment, but had been given no other treatment. His general condition is likewise good.

The prognosis seems to be good in our family, similarly as in the family reported by Schaar [18]. The prognosis of Wiskott–Aldrich's syndrome was formerly regarded as hopeless. However, several reports are now available on patients who are still alive even after several years of illness.

Summary

A family is described with sex linked, essential thrombocytopenia in two generations. In two of the patients the haemorrhagic diathesis was the only sign. Of these, one became symptom-free spontaneously about the age of puberty, while the other became symptom-free in response to splenectomy at the age of 18 years. The three other patients had, in addition to haemorrhagic diathesis, a mild tendency to infection and eczema. One of these became symptom-free after splenectomy, but died later of suprarenal haemorrhage, while the two others were alive and in good general condition at the time of investigation.

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CASE REPORT

Diverse Chromosomal Anomalies in Two Siblings

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A number of examples of different chromosomal anomalies occurring in siblings have been reported. Most of these have been the result of multiple instances of nondisjunction occurring in the same family [1, 3, 4, 5, 8, 9, 15, 17, 18]. The present paper concerns two siblings with chromosomal abnormalities. One of them is a mentally retarded boy with epilepsy and microcephaly associated with an extra small metacentric chromosome. His sister is a typical case of mongolism with trisomy of one of the small acrocentric chromosomes.

Case Records

Family history. The patients' father was born in 1920 and died at the age of 42 of alcoholic cirrhosis. The mother was born in 1919 and is in good health. A paternal uncle died of aplastic anemia at the age of 35. No instances of mongolism, congenital malformations or mental retardation have been recorded among the near relatives of the parents. There is no parental consanguinity nor is there consanguinity in paternal or maternal grandparents. The mother gave no history of serious illness or abortions. She has had only two pregnancies.

Case 1, the first child, a female, was born in 1944 after an uncomplicated pregnancy and delivery. She was born 16 days before term. The birth weight was 2750 g. Typical mongoloid features were noted at birth. At 10 years of age psychological testing (Terman Merrill) revealed an IQ of 30, and at the age of 12 the IQ was 31. At the present time she has short stature, brachycephaly, highly oblique palpebral fissures, Brushfield spots, and an epicanthic eye fold on the left. The ears are low-set, small and prominent. The mouth is usually open and there is macroglossia. The lips are furrowed. The alignment of the teeth is irregular, and the palate is high-arched and narrow. A loud systolic murmur is audible over the base of the heart. The muscles are hypotonic, and the joints are hyperflexible. The hands are short and broad, and the fifth fingers are short and incurved. She has small feet with marked gap between the first and second toes. The palm and finger dermal ridge patterns are typical of mongoloids with a maximum atd angle of 84° for the left and right hands. Secondary sex characteristics are normal.

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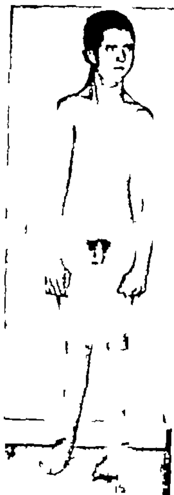


Fig. 1. Case 2 at 16 years of age.

Case 2, the second child, a boy, was born in 1947 after a normal pregnancy and delivery at term. The birth weight was 2510 g and the birth length 50 cm. At birth the head, which was noted to be *small with a* peculiar shape, had a circumference of 33 cm. The facial features, especially the mouth, were small. The chest circumference was 34 cm. During the first three days of life he was sluggish and frequently vomited. On the fourth day after birth there was one episode of vomiting and a small amount of brown vomitus was noted. Platelets were normal (*04,000/mm*) and the prothrombin time was 10 minutes (normal *0–30 sec). A stool guaiac test was negative. The hemoglobin and red-cell count were normal. The white-cell count was 13,900/mm

Because of marked mental retardation, he was admitted to an institution *for mental* defectives at the age of 7 years. Physical examination at that time showed an asthenic physique with long arms and sloping shoulders. The testes were undescended. Quick stereotyped movements of the arms and hands were noted. Psychological testing (Bühler-Hetzer) revealed a development quotient of 22. He was characterized as destructive, very restless and autistic. In February 1955, at the age of 8 years, he had two seizures of –3 minutes duration with facial twitching and decreased consciousness. An EEG showed a generalized, moderately dysrhythmic pattern with abundant paroxysmal convulsion activity breaking through. With Difhydan (Phenantoinum) therapy he became free of fits. In December 1958 the left testis was found to be descended and in February 196* both testes were palpable in the scrotum. At this time it was noted that in states of anxiety there was *increased* muscular tension in the arms and legs which were kept in different stereotyped positions. In December 196., physical examination again showed an asthenic physique with slender bone structure (Fig. 1). The height was 15 cm and the weight 41.4 kg. Craniometry gave the following results: circumference 51 cm, anteroposterior length 15.6 cm, width 13.8 cm and cephalic index 89. The palpebral fissures were straight but somewhat narrow. No epicanthic eye-folds or Brushfield spots were present. The mouth was very small and usually open. The *tongue* was normal. The palate was high-arched and narrow. The teeth were normal. The ears appeared normal. There was a slight thoracic kyphosis. Otherwise the chest was normal. Examination of the heart was negative. The abdomen was normal. The testes appeared smaller than normal. There was a *slight amount* of pubic hair and n axillary hair was present. The muscular tone in the arms and legs was slightly increased. The elbow joints were hyperflexible.

TABLE 1. *Analysis of dermal ridge patterns of Case 2 (according to Beckman et al. [2])*

Types of finger-tip patterns			Occurrence of patterns in different palmar areas		
Digit	Right hand	Left hand	Pattern areas	Right hand	Left hand
I	whorl	radial loop	Hypothenar	+	−
II	radial loop	radial loop	Thenar and I	−	−
III	radial loop	whorl	II	−	−
IV	whorl	whorl	III	+	−
V	radial loop	ulnar loop	IV	−	−
			Maximal atd angle	86	46
			Four finger lines	−	−

X-ray films of the skull revealed slight thickening of the calvarium. Films of the other bones and the chest were negative.

The hemoglobin was 12.4 g% and the white-cell count was 7700 mm³ with a normal differential count. The platelet count was 285,000/mm.

Testing of the clotting factors (prothrombin, proconvertin, Christmas factor and Stuart Prower factor) with the thrombotest [11] showed a coagulation activity of 44% (normal range 70–130%).

Urinalysis was negative. A test for the presence of phenylpyruvic acid was negative and two-dimensional paper chromatography for urinary amino acids was normal.

Analyses of palmar and finger dermal ridge patterns (Table 1) showed a high atd angle on the right palm, a radial loop on the left thumb and on the right fifth finger. These findings are uncommon in normal individuals. The dermal configurations were not of the type found in mongolism or 17–18 trisomy syndrome [2, 13, 16].

Since admission to the institution, he has become quieter and has had better contact with his surroundings. He cannot talk but understands simple commands. He can feed himself but is unable to dress or undress, and he has fecal incontinence and enuresis.

Cytological observations

Karyotypes were obtained from cultured leucocytes from both siblings and the mother. The technique employed was a modification of the method of Moorhead et al. [10]. In addition skin cells were cultured from a biopsy specimen obtained from Case 2 according to the technique used in this laboratory [5]. The maternal karyotype was normal. The results obtained from both siblings are summarized in Table 2.

The leucocytes from Case 1 showed a

TABLE 2. *Summary of chromosome analyses of the patients and their mother*

	Chromosome counts					
	45	46	47	48	Total	Karyotype interpretation
Case 1: Blood		2	33		35	Trisomie 21
Case 2: Blood		1	48		49	Extra small metacentric chromosome
Case 2: Skin		1	38		39	Extra small metacentric chromosome
Mother: Blood			43		43	Normal karyotype



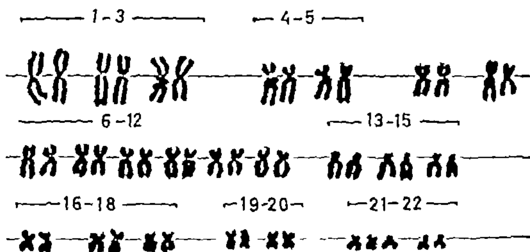


Fig 2. Karyotype of Case 1 There are 47 chromosomes, including a trisomy in the 21–22 group.

modal number of 47 with trisomy in group 21–22 (Fig 2) Both the leucocytes and skin cells of Case 2 showed a chromosome number of 47 with an extra small metacentric chromosome that appeared to be slightly smaller than the small acrocentrics (Fig 3)

Both the buccal mucosa cells and cultured skin cells of Case 2 were negative for sex chromatin.

Discussion

Case 1 is a typical case of mongolism, and the chromosomal abnormality has probably occurred as a result of non disjunction in one of the parental gonads.

The origin of the odd chromosome in Case 2 cannot be determined with certainty from its morphological appearance or from clinical evidence The clinical findings in the present case do not fit into any of the previously described syndromes related to autosomal abnormalities such as (13–15)-trisomy 18-trisomy or 21 trisomy (mongolism) He had however a small mouth and muscular hypertonicity which are frequently associated with the 18-trisomy syndrome

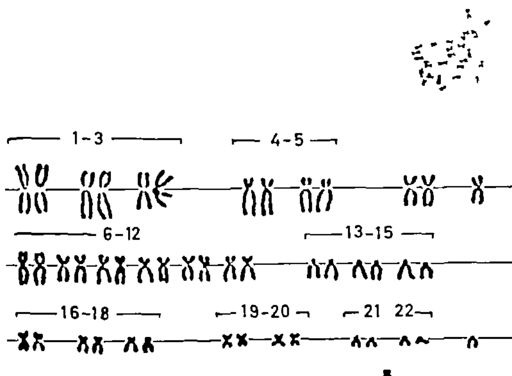


Fig. 1. Karyotype of Case 2. There are 47 chromosomes, including an extra small metacentric chromosome (placed below group 21 22).

The odd metacentric chromosome could be the result of a translocation or a deletion during meiosis. It could also represent an isochromosome for the short arm of a chromosome of pair no 17 or 18 In the better-quality metaphase plates the lengths of the arms of the abnormal chromosome appeared similar to the short arms of no. 18 rather than 17 Isochromosomes are usually the result of misdivisions of the centromere during meiosis (6 7 10 14). Since the patient is sex chromatin-negative it is probable that the extra chromosome does not represent a deleted X, but this possibility cannot be excluded. The chromosomal anomaly could have arisen after the formation of the zygote but in this event one might expect evidence of mosaicism, which was not found.

The chromosomal abnormalities in the two siblings under discussion could be a matter of coincidence or they could possibly be in some way related to each other One cannot say whether the factor or factors responsible for the formation of unbalanced gametes could have been present in either or both parental gonads.

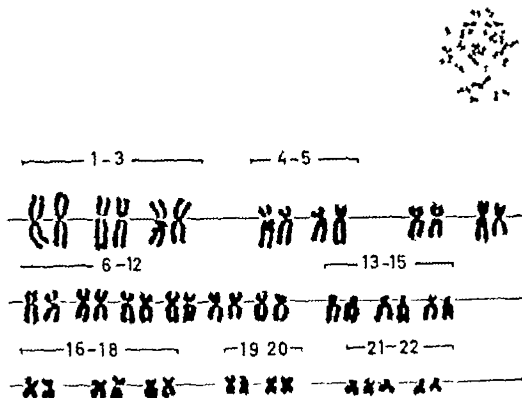


Fig. 2. Karyotype of Case 1. There are 47 chromosomes, including a trisomy in the 21–22 group.

modal number of 47 with trisomy in group 21–22 (Fig. 2). Both the leucocytes and skin cells of Case 2 showed a chromosome number of 47 with an extra small metacentric chromosome that appeared to be slightly smaller than the small acrocentrics (Fig. 3).

Both the buccal mucosa cells and cultured skin cells of Case 2 were negative for sex chromatin.

Discussion

Case 1 is a typical case of mongolism and the chromosomal abnormality has probably occurred as a result of non-disjunction in one of the parental gonads.

The origin of the odd chromosome in Case 2 cannot be determined with certainty from its morphological appearance or from clinical evidence. The clinical findings in the present case do not fit into any of the previously described syndromes related to autosomal abnormalities such as (13–15)-trisomy, 18-trisomy or 21-trisomy (mongolism). He had, however, a small mouth and muscular hypertonicity which are frequently associated with the 18-trisomy syndrome.

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CASE REPORT

Aortic Hypoplasia

by OLE EKLÖF, DARCY O. ILHA and PER ZETTERQVIST

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Although well known to pathologists since the middle of the 18th century isolated hypoplasia of the aorta still remains a rather uncommon clinical entity. It is found more commonly as a coexisting anomaly in congenital cardiovascular disease [12, 16]. However, in view of the relatively few cases reported and the unusual diagnostic problems presented by these patients it seems worth while reporting two cases studied by us, one of them associated with supravalvular aortic stenosis, and each associated with mental deficiency.

Hypoplasia of the aorta has been defined in various ways, some authors including both luminal constrictions of peripheral arterial vessels [1] and segmental aortic involvement [13] in this term. In our opinion, however, the term aortic hypoplasia should be restricted to only those cases in which the aorta is small in caliber along its entire extent. This definition does not include the coexistence of other vascular anomalies.

Normal measurements of the aorta vary according to age, sex and constitution [8, 16]. Arvidsson [3] recently correlated cross-sectional areas of the thoracic aorta in infants and children, studied by angiocardiography, to the body surface area, finding a linear relationship.

Supravalvular aortic stenosis is another relatively rare condition sometimes observed in conjunction with aortic hypoplasia [12]. In these cases there is an additional constriction of the aortic lumen just above the aortic valve. The morphological criteria for the diagnosis of supravalvular aortic stenosis have recently been enunciated by Peron [15] who found the true supravalvular stenosis both grossly and microscopically akin to coarctation of the aorta.

In many cases of aortic hypoplasia and supravalvular stenosis the patients are both physically and mentally retarded [4, 6, 16, 17]. Some of the mentally retarded patients have a characteristic facial appearance which in some cases constitutes a further clue to the correct diagnosis. Nevertheless, similar appearances have been found in other cases without aortic hypoplasia or supravalvular aortic stenosis.

The main clinical finding in aortic hypoplasia has in many cases been an elevation of the blood pressure, usually ascribed to the reduced capacity of the aorta. Pressure differences present in the limbs may be explained by involvement of the appropriate arteries. Some patients reveal symptoms of congestive heart failure with reduced pulse pressure. The signs and



Fig 5. *Case 1* Conventional chest roentgenograms showing moderately increased heart volume. (A) Frontal view, (B) lateral view Both the left atrium and ventricle are enlarged. There is narrow *vascular pedicle The aortic arch is not identifiable.*

At the initial examinat on in 1958 the girl was found to have facial asymmetry (Fig 1) strabismus, loose ligaments, and palatum ogivale Inspection of the precor dium revealed a slight bulge and a diffuse area of maximum impulse in the fifth and *sixth intercostal spaces outside the mid* clavicular line The cardiac impulse was in creased in breadth and heaving A systoli thrill was palpable over the aortic area, man brium, and vessels of the neck.

A harsh systolic murmur of a stenotic" type was heard and recorded (Fig 3), having it maximum intensity over the aortic area and manubrium with transmission to both sides of the neck. The second sound was single and well ud ble though not accentua ted over the second intercostal space bilater ally Blood pressure: Right arm 130/105 left arm 105/85, legs 120/80. The electrocardiogram indicated marked left ventri cular hypertrophy (Fig 4)

Conventional chest roentgenograms (Figs. *5A and 5B*) showed a pathologic increase in heart volume Both the left atrium and ventricle were moderately enlarged. The as cular pedicle was narrow The aortic arch was not identifiable The pulmonary veins were probably slightly dilated.

A second examination was made in 1962. No significant alterations had occurred on the ECG The heart volume had increased *further The patient had been treated* ith digitalis during the last three ears.

Angiocardiography using a transseptal technique was carried out with injection of the contrast medium into the left atrium (Figs. 6 A, B, C and D) There was reflux into the veins of the left upper lobe The left atrium was moderately enlarged and there was a considerable dilatation of the left ventricle A moderat hypertrophy of the left ventricular wall was present. The possi bility of low sub- al ular stenosis could not be ruled out due to the fact that it was not possible to record the pressures t this level. The aortic al ular ring and the aortic leaf let appeared normal. Approximately t o centimeters above the alvular plane there was a *circular constriction I the aortic* lumen almost one centimeter in length. The caliber of the aort gradually enlarged abo

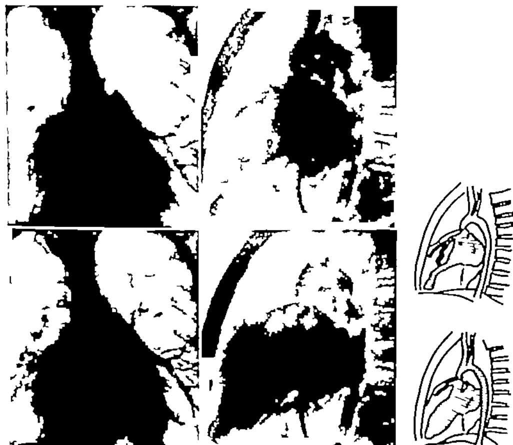


Fig. 6. *Case 1* Angiocardiography with contrast injection int the left atrium. (A and B) Frontal and lateral views in systole; (C and D) frontal and lateral views in diastole. Dilatation of the left atrium and ventricle is confirmed. The aortic valvular ring and the aortic leaflets are normal. About 2 cm above the valvular plane there is luminal constriction almost 1 cm in length. The aortic lumen gradually enlarges above the stenosis but remains subnormal in its entire length.

the stenosis but remained smaller than normal. The aortic arch was low and the vessels arising from its convexity had a long intrathoracic course There was no bnormality at the origin f these essels. There was considerable dilatation and tortuosity of the anterior descending branch of the left coronary artery The right coronary artery was moderately dilated and tortuous. The circumflex branch of the left coronary artery was only partially seen.

It was concluded that the patient had a supra valvular aortic stenosis complicated by a slight hypoplasia of the aorta, possibly in association with low sub-valvular stenosis f the left entricle

At *operation* (Dr Cid Nogueira, PA) in Dec 1962, the systolic pressure in the left ventricle was 190 mm Hg with a recording in the left femoral artery of 115 mm Hg (Fig 7*a*). The narrowest aortic segment was located 1 2 cm bove the valvular plane The aortic wall was obviously thickened in the entire ascending part Following resection of the stenotic part the systolic pressures in the left ventricle and the femoral artery were normal and equal (Fig 7*b*).

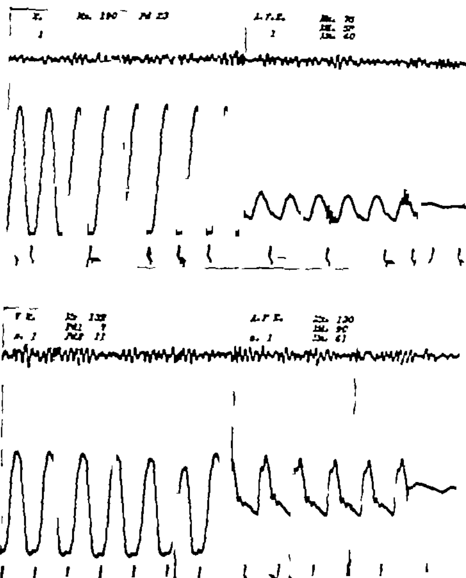


Fig 7 *Case 1* Pressure recordings from the left ventricle nd femoral artery ( ) Before and
following surgical correction of the supra alvular aortic stenosis.

Case 2

J Ö., a boy born in 1957 The patient somatic and mental development had been slow He began to walk at 17 months. At the examination in 1962 the i.q. was around 60 At three months of age a harsh syst lic murmur was detected over the base f the heart. At about one year of age strabism was noticed and t the age of 1½ yea bilateral hyperopia was diagnosed The p tent had lways had ncreased fatigabilit and breathlessness on effort

The physical examination in 1962 show a peculia appearance with a prominent inter

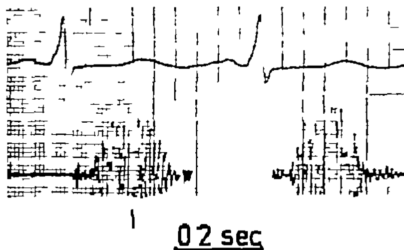


Fig 8. Case 2. Phonocardiogram obtained over manubrium of the sternum.

frontal suture suggestion of a saddle nose, and for the most part an open mouth (Fig 2). Inspection of the precordium was normal. The apex beat was not palpable A grade V systolic murmur and systolic thrill were found over the manubrium of the sternum and bilaterally over the first two intercostal spaces and along the common carotid arteries. It was distinctly higher-pitched over the manubrium than along the carotids where it had a rumbling character A phonocardiogram showed the murmur to be typically diamond shaped over the manubrium (Fig 8) and also confirmed that it was of higher frequency in this place than elsewhere. The second sound over the pulmonary

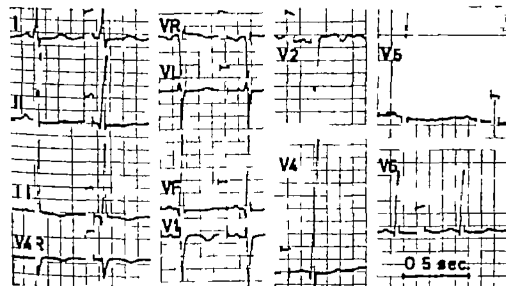


Fig 9 Case 2 Electrocardiogram.



Fig 10. *Case 2* Conventional chest roentgenograms showing normal heart volume (A) Frontal view; (B) lateral view Slight enlargement of the left atrium is noted. The aortic arch is not identifiable

area was single and neither accentuated nor reduced in strength. Blood pressures were: Right arm 150/90- left arm 140/90- legs 130/80 mmg Hg The ECG showed typical features of left ventricula hypertrophy (Fig 9)

Conventional chest roentgenograms showed a normal heart volume (Fig 10 A and B) The left atrium bulged slightly posteriorly indicating some enlargement of this cavity The aortic arch was not distinguishable The pulmonary vessels were normal.

At heart catheterization repeated attempts at puncturing the femoral artery failed, probably due to th small caliber of the patient systemic arteries. Instead, right heart catheterization was performed, showing normal pressure conditions in both right heart cavities as well as in the pul monary artery

Angiocardiography was performed with injection of the contrast medium into the pulmonary trunk (Figs. 11 A B C and D). The pulmonary arteries were slightly slender and the pulmonary val es were normal The pulmonary veins had the usual distribution and drained into the left atrium. This cavity was slightly enlarged. The left entricle was of normal size and configuration. The ventricular wall was moderately hypertrophic. The aortic valvular ring was of normal size The aortic leaflets were of ordinary thickness and mobility No obvious changes of the sinuses of Valsalva were present The aortic lumen was markedly reduced in its entire extent with caliber nowhere exceeding half the normal. The aortic arch was short and the vessels arising from its convexity had a very long intrathoracic course There were no abnormalities of the emergent vessels. The anterior descending branch of the left coronary artery wa moderately dilated and sinuous. The right coronary artery wa mildly tortuous and dilated. The circumflex branch of the left coronary artery was not visualized.

It was concluded that the patient had general hypoplasia of the aorta without stenosis of the aortic orifice or supra valvular aortic stenosis.

Discussion

In the presence of a characteristic stenotic murmur a peculiar facial appearance as described above strabismus, mental re tardation, and a high systoli blood pres-

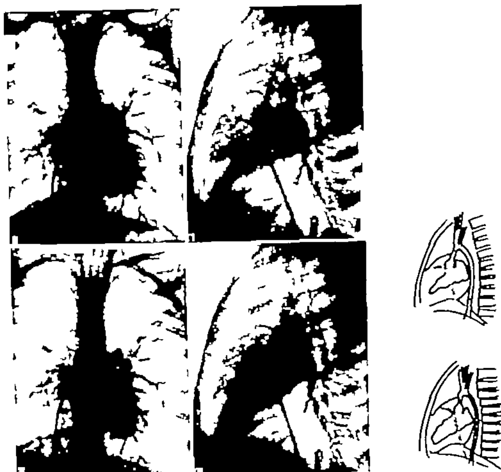


Fig. 11. *Case 2.* Angiocardiography with contrast injection into the pulmonary trunk. (A and B) Frontal and lateral views in systole; (C and D) frontal and lateral views in diastole. The pulmonary veins drain int the left atrium which is somewhat enlarged. The aortic valves are normal The aortic lumen is markedly reduced in its entire extent.

sure the possibility of aorti hypoplasia should be kept in mind.

The aetiology of this syndrome is un known [ ]. There is nothing to indicate an environmental cause or a single Mendelian gene as the origin. The combination of mental retardation with a relatively con stant set of malformations indicated the possibility of an underlying chromosomal aberration [14]. In one of our cases, exa mined in this respect, no bnormalities could be found.

The histological picture of the hypoplastic orta is characterized by under development of the elastic muscular tissues but no other characteristic feature is present [9].

Varying degrees of reduction of the or tic caliber may occur as a disease *sui generis* but is more often seen as a part of a complex cardiovascular malformation [1].

Elevation of the systolic blood pressure has been registered in several cases of aor

tie hypoplasia but the diagnostic value of this sign has not been emphasized. The abnormal pressures noted should be regarded as a consequence of the diminished distensibility and capacity of the aorta. It is, however still a matter of speculation that due to an increasing peripheral arterial resistance, in time the diastolic pressure as well will rise giving the picture of established hypertensive disease The different pressures recorded in the limbs should be attributed to abnormalities in distribution and caliber of the regional arteries

Since in some cases of aortic hypoplasia coexisting anomalies of the pulmonary vascular system have been described [5 13 17] this area should be studied as well, preferably by right heart catheterization and separate serial right ventricular or pulmonary angiography

The association of aortic hypoplasia and supravalvular aortic stenosis is not infrequent [1–]. In most cases the supravalvular stenosis is due to an exaggeration of the transverse infolding or plication of the aortic wall at the upper limits of the sinuses of Valsalva [11] This infolding of the media capped by slight intimal thickening may give rise to variable degrees of constriction of the aortic lumen. Although a circumferential involvment of the vessels is the most common finding, there are cases with only a supravalvular membrane causing impairment to the aortic flow [6 7] Still there are cases with a "free floating membrane or band extending across the aorta in the supravavular region causing no functional impairment at all [15 19] Usually the aortic leaflets are quite normal but in some cases one leaflet may adhere to the aortic wall [10] and may in this way compromise the blood flow into the coronary arteries [18].

Differentiation between existing stenotic lesions in the aortic valve cannot be made on clinical grounds alone The anatomical diagnosis rests on the angiocardiographic findings.

The prognosis in the individual case with aortic hypoplasia depends on the degree of narrowing of the aorta and the condition of the myocardium. In cases with extreme reduction in caliber the malformation causes a strain on the left ventricle resulting in left ventricular hypertrophy and heart failure [16]. So far there is no known therapy In supravalvular stenosis on the other hand, surgical correction is possible and the results have been encouraging

Summary

Two children are reported, presenting the syndrome of aortic hypoplasia, mental retardation, strabismus, and a *characteristic* facial appearance. The cardiologic findings in each case included a systolic murmur over the aorta and evidence of left ventricular hypertrophy In one case a moderate general hypoplasia of the aorta was associated with marked *localized* stenosis of the supravalvular aortic portion. Following surgical resection of the stenotic area the pressure conditions were normalized In the other case the entire aorta was hypoplastic, and as a consequence of its reduced capacity an elevation of the systolic blood pressure was found in all extremities. The discussion concerns mainly the anatomical variations of the aorta present in this syndrome and the significance of selective angiocardiography for their detection

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SUMMARY OF SUPPLEMENTS

Cardiomyopathy in Friedreich's Ataxia

With Studies of Cardiovascular and Respiratory Function

by CLAES THORÉN

(*Supplement 153*)

The main purpose of the investigation is to study the presence and nature of the heart disease in Friedreich's ataxia. The clinical features and haemodynamics of the cardiomyopathy and its effect on myocardial function are studied. This is done by means of heart catheterization, determination of circulatory dimensions and lung function, as well as by exercise tests and measurements of the peripheral circulation. The development of the ECG changes and the way in which they are influenced by various functional conditions are also analyzed. In addition, the pathoanatomical background of the heart disease is studied.

The series comprises 66 cases of Friedreich's ataxia, classified into four groups according to the degree of physical handicap. A clinical examination is made of 49 patients, as well as of their parents and sibs. In addition 8 "atypical" cases are examined, and also 34 patients with other neurological syndromes, previously suspected to be Friedreich's ataxia.

A definitely abnormal ECG is demonstrated in about 90 % of the cases of Friedreich's ataxia. No correlation is found between the degree of neurological dysfunction and the degree of heart disease, whereas the latter shows a similarity in sibs. In 5 cases, the heart disease is detected before the neurological manifestations. Signs of cardiomyopathy are present in only one sibship among the "atypical" cases, and in only one of the excluded cases, who had a form of chronic encephalopathy.

Degeneration of the myocardium as well as of certain nervous tissue is suggested to be the result of a single genetic defect. Friedreich's syndrome is therefore proposed as a more suitable name for the disease.

Three neurologically healthy sibs of the patients, as well as one mother, have an obviously pathological ECG, but it cannot be determined whether isolated cardiomyopathy does, in fact, occur in these families.

The most common ECG changes are T wave inversion, signs of left ventricular hypertrophy and extrasystoles. Work on the bicycle ergometer—performed in the wheel-chair cases by a special method devised for cranking—is accompanied by reversion of the T waves in more than two-thirds of the patients. Similar normalization occurs during an orthostatic test in one-third of the cases, and in half of those in which ganglionic block is induced

A follow up study of the ECG shows reversion of the T waves in one third of the cases.

The blood volume and the total amount of haemoglobin are found to decrease with the degree of physical handicap, as a result of inactivity and muscular atrophy The heart volume in relation to the total amount of haemoglobin is on the contrary increased.

The disease is characterized by an abnormally high heart rate despite a low working intensity This is explained by a low oxygen pulse and, as a rule an extremely low stroke volume The filling pressure of the ventricles increases with the duration of the disease.

Despite a low cardiac output in relation to oxygen uptake—whi h applies in the majority of cases—signs of poor oxygen utilization are present, even in the few patients with a more hyperkinetic circulation A disturbed distribution of the cardiac output is suggested as the cause

During work, the increase in cardiac output is generally ordinary in relation to oxygen uptake despite a decrease in stroke volume in several cases.

Studies of the peripheral blood flow by occlusion plethysmography show a reduced flow in the forearm and calf at rest, as well as reactive hyperaemia in correlation to the degree of physical handicap During ganglionic block, an increase in reactive hyperaemia is noted in the wheel-chair cases. Muscular inactivity and raised vasoconstrictor tone are suggested to be responsible for the decreased blood flow A decreased cardiac output is a possible contributory factor

Angiocardiography discloses hypertrophy and rigidity of the walls, of the left ventricle and systolic contraction of the infundibulum of the right ventricle which may contribute to the systolic ejection murmur This is shown by phonocardiographic analysis to differ from a murmur of physiological type

The murmur sometimes disappears with increasing duration of the disease which is explained by a falling stroke volume and more hyperkinetic circulation.

Pulmonary function is studied by static and dynamic spirometry The total lung capacity is within the normal range except in the wheel-chair cases, in which it is slightly reduced, due to a greater degree of kyphoscoliosis Friedreich s ataxia is characterized by a raised residual volume a high ratio of functional residual capacity to total lung capacity and an elevated level of the tidal volume An abnormal rhythmic activity of respiration occurs. A disturbed respiratory mechanism is suggested to be responsible for this pattern, which also contributes to the speech disturbances. The forced expiratory volume in one second is normal, but repeated forced ventilation is impaired Interrupted transmission of proprioceptive afferents from intercostal muscles may explain the essential disturbances in pulmonary function.

Signs of generalized myocardial hypertrophy and reticular fibrosis are present in all four cases in which autopsy is performed. Subendocardial fibroelastosis and mural thrombosis are seen in the left atrium. All four cases display subintimal fibrotic hyperplasia of the large and medium-sized branches of the coronary arteries occasionally with total occlusion. It is pointed out that the pathogenetic consequences of the coronary changes are however obscure.

Acta Pædiatrica 53 390-395. July 1964

PROCEEDINGS OF PEDIATRIC SOCIETY

Norwegian Pediatric Association

Meeting Febr 22 1963

S Halvorsen Regulation of the Erythropoesis in the Newborn

Publ. *Acta Pædiat (Stockh) 52* 1-5 1963.

D Skyberg Generalized Neonatal Herpes Simplex

A boy born at term, falls ill on the fifth day of life with rapidly increasing listlessness, anorexia and cyanosis. Dyspnea with hepatic congestion and pulmonary rales develop. The condition is treated as sepsis with heart failure the infant dies following two and a half days of illness. Autopsy revealed focal necroses in the liver and adrenal glands together with a necrotic ulcus in the esophagus near the cardia. In the necrotic borders were demonstrated typical intra nuclear inclusion bodies of the type Cowdry A together with morphologic changes similar to those described in reports on fulminant virus infections. Culture of liver tissue on HeLa cells gave growth of herpes simplex virus. The mother's blood did not contain herpes antibodies and this had made the unprotected infant susceptible to the in fection The source of contamination was not demonstrated.

The diagnosis of generalized neonatal herpes simplex has not previously been made in Norway

Meeting March 29 1963

Al Seip Hereditary Hypoplastic Thrombocytopenia

Publ. *Acta Pædiat (Stockh), 52* 370, 1963.

Arne Kåss Tuberous Sclerosis

A case of tuberous sclerosis has been observed in the Pediatric Department in Vestfold County Hospital. There is nothing significant in the family history The preg nancy was uneventful. The patient is a bi ovular twin and up till now the other twin has presented no abnormal symptoms. A few weeks old, the patient presented psychomotor retardation. At four months of age an exanthema typical of adenoma sebaceum was found with marked papillary atrophy; pneumoencephalography revealed intra ventricular tumors in the frontal and temporal horns. Biopsy of skin from the face showed changes compatible with the diagnosis. Prednisone treatment kept up over several weeks gave no sure clinical improvement, and renewed pneumoencephalography revealed unaltered conditions. Up till now the patient has presented no epileptic symptoms. The dementia was markedly progressive and at 8 weeks of age the patient was transferred to a mental home

Øystein Aagenæs. Hereditary Non-spherocytic Hemolytic Anemia

The patient, E. S is the second of two sisters. The mother has a tendency to anemia, particularly during pregnancy and has frequently had iron treatment. The now 4 year-old sister had icterus in the neonatal

period and was anemic at 2 months of age The anemia disappeared at bout 1 year of age following iron treatment.

Pregnancy and birth were normal. Birth eight was 3210 g. At two weeks of age the patient was slightly icteric and pale Four weeks old she was admitted to the Pediatric Department of the University Hospital because of anemia. Blood tests: Hemoglobin: 25%. Saturation index 1.0. Coombs reaction: Negative Reticulocytes: 14.%. Thrombocytes: 180 000. Leucocytes: 8600 with normal differential count. There was anisocytosis but no spherocytosis. Bone marrow: Lively normoblast erythrocytopoiesis. Serum iron: 48 gamma per cent. Osmotic fragility: Normal. The life span of the erythrocytes (Cr^{51}) was reduced; it was 23 days in the patient and 28 days in the mother

Preliminary examinations have given no explanation for the shortened life span of the erythrocytes. Using the Dacie method, autohemolysis was found to be markedly increased and the hemolysis was not hampered by addition of glucose (Dacie type II). Glucose-6-phosphate-dehydrogenase gluta thione, glutathione reductase and pyruvate kinase are found in normal amounts in the erythrocytes (Dr Borresen, Central Labora tory Oslo University Hospital). Hemoglobin lectrophoresis of the infant blood at three months of age shows that about 30% of her hemoglobin is made up of fetal hemoglobin. This is supposed to be secondary to the hemolysis, but will be followed. The serum haptoglobin content is reduced (*0 mg%), probably also secondary t the hemolysis.

The patient was initially given two trans fusions. Her hemoglobin has later risen spontaneously to 75–80%.

Meeting May 24–25 1963

J Marstrander Epidemic Pneumonia in Infants

During the period December 1962 through February 1963, 80 patients under 2 years of age were admitted to the Pediatric Depart ment Ullevål Hospital because of bronchopneumonia. The clinical picture was approximately the same in all cases, with typi cal symptoms of what has also been called acute bronchiolitis, capillary bronchitis, malign tracheobronchitis, and interstitial pneumonia. Rales over the lungs were present in all patients for shorter or longer time especially when the stage of acut respiratory distress was past. Of the 80 patients, 53 were under 6 months of age 19 were from 6 t 12 months old and 9 from 1 t 24 months old, an ge distribution which tallies with that indicated in previous publications. The younger the patient the more severe was the disease The findings t bacteriological examinations were not uniform. Only 32 patients were f brile. Blood examinations revealed leucocytosis in 24 of the 80 patients. These findings suggest a virus etiology an assumption borne out by the fact that the disease usually occurs during the winter months when the incidence of colds and respiratory infections in the popu lation is t its highest Such infections were present in the immediat surroundings of more than half the patients. Blood sampling for demonstration of virus antibodies in the serum was carried out on 41 patients. The complement fixation reaction to Parainfluenza III showed definit rise in 4 cases, and in 3 cases a rise in the titre to Respira tory Syncytial virus.

All patients were treated with antibiotics, in most cases with penicillin-chloramphenicol, a therapy generally recommended because of the considerable chances of bacterial superinfection. However in our experience the most important is treatment with oxygen vapor and, if necessary with digitalis in patients with severe respiratory complica tions. The treatment should be instituted at the earliest possible moment in which case the prognosis is good. In the present ma terial 3 patients died. They were all under 6 months of age and all had cardiac complica tions in the form of myocarditis or peri carditis.

Ragnhild K1 7 Variant of Maple Syrup Urine Disease

(Will be published in *Acta Pædiat (Stockh)*, 53 1964)

Harald M Svendsen and Eyvind Treterås Neonatal Physiologic Proteinuria

Transient occurrence of increased protein excretion in the urine was considered an established fact until 195 when Doxiadis and co-workers published a study in Lancet claiming that the so-called physiologic neonatal proteinuria is nonexistent. The authors maintain that in reality the alleged proteinuria is a misinterpreted demonstra tion of urates. As there seemed t be some room for doubt it was decided to undertake a renewed study of neonatal albuminuria.

From 36 healthy newborn infants were collected 107 urine samples in the course of the first second and third days of life In all cases pregnancy and delivery had been uneventful, spontaneous vertex presentation and in no case had there been signs of pre- or postnatal asphyxia. Qualitative protein demonstration was done using albustix strips and H ller test and quantitative rotein determination using the Clos me- l. By this means it was possible to demonstrate a urinary excretion f protein of more than 20 mg% in about 40% of healthy newborns on the first d y of lif This albuminuria was transient and had disappeared after the third day of lif in the majority of infants. Thus physiologic proteinuria in the neonatal period seems to remain a fact

K F Stoa Excretion of Estriol in the Urine in Newborn Infants with Congenital Dysplasia of the Hip

The excretion in the urine during the first three days of life of estriol and alpha ketolic estrogens has been determined in *0 new born infants. Eight of the infant presented normal findings, while Ortolani sign was present in 1 During the first two days of life the excretion of estriol was about *00 microgram in 24 hours after which it fell rapidly There was no demonstrable dif ference in estriol excretion between the t o groups of newborns.

Andrén & Berglin (1960 1961) maintain that the metabolism of 17 beta-estradiol is reduced in infants with hip joint dislocation. This finding was not borne out by the present study Only traces of 17 beta-estradiol were demonstrable in the urine in both groups of infants, finding which is in accordance with observations on normal newborns (Diczfalusy & co-workers, 1957). The t o alpha-ketolic estrogen metabolites 16-alpha hy droxy-estrone and 16-oxo-17 bet -estradiol have been demonstrated in urine from new borns for the first time There seems to be a tendency to higher values for these steroids in the group comprising Ortolani-positive infants than in the normal group. The dif ference is not, however statistically significant

P J Moe S Refsum, Jr., O Knutrud Multicystic Kidney

Multicyst c kidney seems to be the most frequent cause of the diagnosis of abdominal tumor in the neonatal period and may occur together with other congenital deformities, especially esophageal atresia. Multicystic kidney is often confused with polycystic kidney The latter is, however bilateral recessively hereditary condition, while family incidence has not been established in any of the 70 cases of multicystic kidney reported in the literature

Four newborn infant with multicystic kidney have been treated in the University Clinic of Oslo in the course of 5 months. Preparations from these patient showed conglomeration of thin walled non-communicating cyst containing a thin yellowish fluid. In three of the specimens fine passages led from the separate cysts t the hilus where a rudimentary blindly-ending ureter was present in two of the cases. Microscopic examination revealed areas of renal tissue

with primitive glomeruli and tubules, hyaline cartilage (dysplastic renal tissue) and thin walled cysts and passages lined with a low cubocylindrical epithelium. There was no sign of stratified epithelium from the renal pelvis, calyxes, or collecting tubules. Bundles of peripheral nerves and areas with thin and thickwalled vessels were also observable

An injury to the fetus which has hampered or stopped further development of the ureter on the 28th to 32nd day of the organogenesis may be a possible explanation of the condition. The metanephric kidney may possibly have been induced by the Wolffian duct or by the nervous tissue of a primitive mesonephric organ. The cysts may have developed because of lacking outlet from primitive, still functioning nephrons.

K Halvorsen, R Nordhagen, J Olsen Rh-erythroblastosis and ABO-incompatibility

Reference was made t a study by Lebech & Bagger Hansen published in *Ugesl Læg* The authors maintain that ABO incompatibility between the fetus and the mother intensifies the effect of the Rh-antibodies from the mother Treatment of material as well as the conclusions drawn are misleading

The own material consist f 118 cases of Rh-erythroblastosis admitted t the Pediatric Department in Bergen in 1961 and 1962 Serological examination was carried out in the Blood-typing Laboratory of Bergen. Four deaths occurred and in all cases there was compatibility with regard t the ABO system. Two of the deaths were due t other causes than the hemolytic disorder The incidence and severity of Rh-erythroblastosis was higher when there was ABO-compatibility between the blood groups of fetus and mother There was also parallelism between the Rh-antibody titre of the mothers and of the degree of reaction t direct Coombs test in the newborns on the one hand and severity of disease in the infant on the other

L. Wirsching J . Eye Symptoms in Acrodermatitis Enteropathica

Report is given of two patient who have developed corneal changes following diodoquin treatment The possibility that the changes may be caused by the drug is discussed. However as the opacities receded together with the other symptoms under increased diodoquin medication there is every probability that the corneal changes are part of the pathological picture and an, up to now unknown symptom in acrodermatitis enteropathica.

(Published in *Acta Ophthalmolog 40* 367 1962)

P A Skogseth. Congenital Hypogammaglobulinemia

Three patients with hypogammaglobulinemia are described. Two are believed to be true congenital hypogammaglobulinemias, while the third might be "transient physiologic hypogammaglobulinemia" The latter died t slightly over 7 months of age. In the two other patients gammaglobulin maintenance treatment has had beneficial effect.

Dagfinn Aarskog Cortisol Concentration and Transcortin binding of Cortisol in the Plasma f the Cord nd the Maternal Plasma after Birth

Cortisol concentration and transcortin binding of cortisol in cord plasma from 9 normal infants born t term, 9 prematures, and 11 infants with diabetic mothers have been studied. There was no significant difference between the three groups in the cortisol concentration of the plasma, neither with reference t cord values nor maternal values. The ratio of maternal value t cord value ranges from 2 1 and 5 1 The transcortin-bound cortisol fraction was higher in the mother than in the infant in all cases. The conjunction was significantly greater in full term infants than in prematures and

there was good correlation between transcortin binding and the gestational age of the infant The non transcortin bound, biologically active cortisol concentration was the same in mother and child. This cortisol fraction was significantly higher for prematures than for full term infants. In infants with diabetic mothers the transcortin binding and the concentration of non transcortin bound cortisol were judged to lie within the limits of what could be expected according to gestational age

Karl W Wefring Serum Bilirubin Values in Newborn Infants Following Prophylactic Vitamin K Administration to the Mothers

Menadione may cause hyperbilirubinemia when given in large doses to newborn infants and the same has been observed when the mothers have received large intravenous doses shortly before delivery The object of the present study has been to examine whether a medium-sized dose of menadione perorally administered prior to delivery may lead to higher incidence and higher degree of icterus in the infants. A dose of 20 mg vitamin K was given 24 hours before delivery and the degree of jaundice was recorded on the fourth day of life. One group of mothers had used vitamin K for several days or weeks prior to delivery

Findings: A total of 728 infants has been examined. The mothers of 372 of them had received no treatment while the mothers of 356 infants had been given menadione In the non-treated group 20 infants (5.37 %) had a serum bilirubin value of more than 15 mg% and mean value was 6.8 mg %. The corresponding figures for the treated group were 17 (4.8%) and 5.75 mg%. There was no significant difference between the two groups as regards incidence of infants with serum bilirubin values of above 15 mg %, whereas the mean value was significantly lower for the treated group. This was more pronounced with reference to the group of mothers who had been on menadione for a longer period, mean value being 5.03 mg %. The reason for this difference is not clear but at least one may conclude that a dose of 20 mg menadione given to the mother shortly before delivery does not lead to higher incidence or degree of icterus in the newborn infant.

(To be published in *J Pediat*)

Meeting Oct 4 1963

Kj B Rootwelt and Jon Steen Johansen. Goiter and Hypothyroidism in Children Due to Defect in the Synthesis of Thyroid Hormone

Three children with defects in the synthesis of thyroid hormone have been examined in the Pediatric Department Rikshospitalet Oslo. The first patient a boy was 1 years old when examined The family history was non-contributory He was retarded and had typical signs of myxedema. The presence of goiter at birth has not been established but he developed goiter later The I[131] uptake in the thyroid gland was increased and fell steeply following perchlorat intake The case has accordingly been classified as a failure to form organic iodine. The second patient was a 5½ years old girl. The parents were second cousins, and several members of the family had thyroid disorders. She had a moderate goiter at birth, but showed no signs of hypothyreosis. Thyroxin medication was started at the age of 6 months. She developed normally From the age of 5 years she had increasing goiter There was a discrepancy between PBI and BEI The goiter gradually disappeared when the dose of thyroxin was increased. The case was classified as defect in thyroglobulin synthesis or proteolysis. The third patient was a 4½ years old girl Several members of the family had goiter She had a large goiter

at birth which was first believed to be a lymphangioma, but later diagnosed as large thyroid gland. The development was normal except for a slight retardation in bone age On thyroxin treatment the goiter disappeared. This case was also classified as a defect in thyreoglobulin synthesis or proteolysis.

Meeting Nov 22 1963

Lei R. Gjessing: Chemical Diagnosis f Neuroblastoma

(Published in *Scand J Clin Lab I vest 15* 1963.)

C Ofstedal Acute Leukemi in Children

In the period 1955–1962 sixty four cases of acut leukemia have been diagnosed in the Pediatric Department, Rikshospitalet, Oslo. Seven cases were for different reasons not treated. Steroid were used initially in almost all cases and were lso used during later exacerbations and in the terminal phase of the disease. As maintenance therapy amethopterin and 6-mercaptopurine were used. Complete or incomplete remissions were obtained in 70 % of the treated cases. The mean survival time in the cases with complete remissions were 14.5 months from the start of the symptoms, the median survival time 11.5 months. The mean survival time for all patients was 10.0 months from the start of the symptoms and the median survival time 7.5 months.

S Halvorsen, Osl

NEW BOOKS RECEIVED

Books received by *Acta Paediatrica* are acknowledged in this column. Selected books will be reviewed in subsequent issues space permitting

Proceedings of the Second International Congress on Mental Retardation. Part I Organic Bases and Biochemical Aspects of Imbecil ty Part II Psychological and Sociological Problems in Imbecility Drug Treatment S Karger AG Basel, 1963 S.Fr 100.

R. Haubrich (ed): *Klinische Röntgendiagnostik innere Krankheiten. Band I Thorax* Springer Verlag, Berlin Göttingen Heidelberg, 1963. DM 220.

Jean Cord er Contribution à l'étude de l'épidémiolog e de la débilité mentale Université Libre de Bruxelles, 1963.

G Jentschura E Marquardt and E M Rudel Behandlung und Versorgung bei Fehlbildungen und Amputationen der oberen Extremität. Georg Thieme Verlag Stuttgart 1963. 136 pages, 107 figs. DM 22.50

H L. Vis Aspects et Mécanismes des Hyperaminoacidurie de l'E fance Recherche sur le Kwashiorkor le Rachitisme Comm et le Scorbut. Editions Arscia S.A Brussels, 1963. 3 5 pages. Numerous figs. Fr B. 540.

Sydney S Gellis and Benjami M Kagan. Current Pediatric Therapy W B. Saunders Company Ltd., London, 1964. 47 pages. £5 12s.

A. W Wilkinson. Recent Advances Pediatric S rgery J & A. Churchill Ltd., London, 1963 306 pages, 75 fig 50 s.

P Royer R Habib and H Mathieu Problèmes actuels de néphrologie i fantile Editions Médicales Flammarion, Paris, 1963.

R. Wenger Endokardfibrosen. Georg Thieme Verlag Stuttgart 1964 DM 30

Herbert G B rch (ed): Bra Damage in Children the Biological and Social Aspects. The Williams & Wilkins Compan Baltimore U.S.A., 1964 199 pages. $ 5.95.

tional Research Institut of Mother and Child, Warszawa, Poland Research Reports, vol I. 1963

Acta Pædiatrica 53: 397–400. July 1964

BOOK REVIEWS

H Loeb Contribution à l'étude du mét bolisme énergétique de l'enfant

Editions Arscia S. A., Brussels, 1962. F B. 350.

The main part of Dr Loeb s studies deals ith the intravenous glucose tolerance test which has been performed in a large number of normal infants and children. In these tests, blood glucose is determined every fourth minute for thirty tw minutes follow ing the intravenous injection of 0.66 ml/kg of 50% glucose solution. The rat of disappearance (K) was found to be higher in most infants and children than in adults. During the first months of life, however K was pproximately the same as in adults. The findings show the reproducibility and accuracy of intravenous glucose tolerance tests. It is regrettable, however that the unspeci fic reduction method for glucose determination was used instead of some of the newer enzyme methods. The author has also stu died the effect of repeating the glucose test and that of administering insulin or tolbuta mide before the test It can be noted, that the well known Straub-Traugott effect could not be demonstrated. Among the results of the studies of pathological cases, the finding of increased K values after leucin dministra tion in leucin-induced hypoglycaemia seems very interesting In some cases, the disppearance of radioactive glucose was also determined. In addition t the studies of glucose metabolism, the author also made in vestigations of free fatty cids and of some keto acids. Compared to normal values in the adult children were found to have higher concentrations of free fatty acids and of α-ketoisocaproic cid.

Lars Wranne Uppsala

P Royer H Mathieu and R. Habib Problèmes actuels de néphrologie infantile

Éditions Médicales Flammarion, Paris 1963. F 98.

The international courses held t the "Centre International de l'Enfance in Paris have become well-known and appre ciated institutions for postgraduat educa tion in the paediatric field. In 1961 a course conducted by Pierre Royer was devoted t the nephrological problems of infancy and childhood. The communications and surveys given at this occasion have now—probably after some expansion—been collected in a volume. It should be pointed out from the beginning that this is a valuable book, both for the general paediatrician and the one with special interests in nephrology

The book begins with chapter on methods for exploration of the tubular functions. Another chapter—covering over 100 pages—is concerned with different kinds of renal disease characterized by tubular disorders. The presentation has in many instan ces been condensed in instructive tables giving the essentials of the disease under discussion. The study of the functional ca pacity f the renal tubules may be elucidating not only in primary renal disease, but also in other disorders where it often can give valuable information bout the nature of metabolic defects. This survey is a good introduction int this field, which merits keen interest by the paediatrician.

Two chapters are concerned with chronic nephropathies with haematuria. The uthors stress the often overlooked fact that although the histological picture t necropsy in such cases often is uniform, histological examination of material obtained by renal biopsies d ring the early stages, suggest

that "chronic nephritis" probably is com posed of many different disease entities.

The authors have restricted themselves to the field of "nephrology" and are only superficially touching upon paediatric urology This necessary limitation has, alas, also to some extent involved the presenta tion of acut uncomplicated pyelonephritis. This disease being the most common, and probably the most important single renal disease had perhaps merited a more com plete presentation. The limited space devoted to urinary tract infections has, however been well used and the authors have time to stress two very important factors in the management of acute pyelonephritis: relapse *is often asymptomatic (and often without* cellular findings, it could have been added): good care of patients with acute infections demands repeated checks during long time perhaps during several years.

The last part of the book is concerned with therapeutical problems, dealt with in an instructive manner Especially should be mentioned the chapters giving practical *guidance for diagnostic and therapeutic* acting in patients with chronic renal failure with chronic proteinuria, with pyuria and w th arterial hypertension.

There are several monographies in the l of nephrology but none devoted espe cially to the often specific problems met with in paediatric patients. We should therefore be thankful to the authors—who themselves earlier have made valuable contributions in paediatric nephrology—for the endeavour to cover this lack. The book is easily read, filled with relevant facts and equipped with a usable index. It is a pleasure to recommend the book, which will meet many demands f paediatricians caring for infants and children with renal disease

Jan W berg Göteborg

F J W Miller R M E. Seal a d M D Taylor: Tuberculosis in Children

Six hundred pages including 7 pages index 119 figures and 6 colorplates. J & A Churchill Ltd. London. 1963. Price £6

This book is produced at the well-known pediatric workshop of the late Professor James Spence clinic in Newcastle upon Tyne This fact fills the reader with great expectations and these are more than ful filled. In general the authors are on the same line as pediatricians in Scandinavia regarding the conception of tuberculosis in childhood. The present reviewer will make only a few comments.

Account is given of experiences of tuber culosis in children up to 15 years of age in North Eastern England and in South Wales. Half of the children were under 5 years of age when admitted. The clinical material comprised 1200 children who since 1947 received chemotherapy From 1957 admissions of children to hospital for tuberculosis fell harply and serious clinical manifestations are now quite uncommon. At school entry the incidence of tuberculin positive children is % or less, at 10 years of age 6 and at 1 -13 12 4%. As to the persistence of a positive tuberculin test in not vaccinated hil ren the authors have the same experience as Scandina ian pediatricians, that reversion is rather uncommon. A thorough description of the public health aspects of tuberculosis in childhood and how to manage detection of the infection and to institute prophylaxis, a good survey of various aspects of BCG vaccination, it results and it complications i given. Very minut description is gi en of pleural effusion, its ignif cance and the value of chemotherapy regarding prophyla xis and remote prognosis. Segmental lesions and development of bronchiectasis are discussed in detail. It seems as if especially the latter complications are gi en more atten tion in England than in the Scandina ian countries. One is little surprised at the high number of hildren with pulmonary t berculosis who have been operated upon, more than *00 in the experience of one single phy sician. This must be due to more liberal opinions for surgery than w follow as rule Steroid therapy in conjunction with chemotherapy has been used rather frequently in all forms of t berculosis The experience with this combined treatment has been

satisfactory giving quicker control of fever and toxic symptom, shortening absorption of inflammatory exudate and elimination of organisms and reducing the amount of structural damage by the process of repair Referring t their study of the site of primary lesion in chronic pulmonary tuberculosis the authors maintain that the exogenous re-infection is rare in this age group. As regards erythema nodosum the authors have practically the same experience as w have They believe, however that children who convert with erythema nodosum have a more serious initial illness and a greater chance of complications than those who do not How can this tally with their conclusion that the child with erythema nodosum is actually t an advantage because he is treated with prophylactic chemotherapy? Much prudent advice is given regarding the differential diagnosis and treatment of local tuberculosis. In t berculous meningitis the authors are reluctant t abandon intrathecal therapy complet ly until they are quit sure that the chances of full recovery are at least equally as good as if it had not been given and they add as yet no convincing evidence has been presented and it is unlikely that it could be collected in England"

It has been of great interest t the reviewer t compare the two last published Anglo-saxon books on tuberculosis in children, this British one and the American published by Edith Lincoln and Edward M. Sewell (see *Acta Paediat* (*Stockh*) 53 97 1964). Both are excellent textbooks and treat the disease in childhood somewhat differently complementing one another For us pediatricians in countries with a high standard of public health these books represent historical reading of great interest. For doctors in developing countries they give an amount of fact and experiences that must be of great help in their endeavour t fight this serious disease in their countries.

Arvid Wallgren Stockholm

W H Hitzig: Die Plasmaproteine in der klinischen Medizin. Ergebnisse spezifischer Bestimmungen mit besonderer Berücksichtigung immunochemischer M thoden

Springer Verlag, Berlin–Göttingen–Heidelberg 1963. 109 fig. 291 pp DM 58.

The study of serum proteins has been expanding so rapidly during the last few year that it has become quit impossible for the non-specialist to keep informed bout the many new techniques and clinical findings, which are being presented t short intervals. The author of the present book has been engaged actively in research within this field for several years, during which time he has been "Privatdozent at the Kinderspital in Zürich.

The present monograph is a very good introduction to this complicated subject because it explains both the theoretical background, the technical procedures and the clinical findings. In the first chapter the modern concept of the physio-chemical structure of protein is explained, including description of the formation of antibodies, antigens and the concept of immunology Then follows a brief account of the many new and old methods by which the different protein fractions can be measured. Special emphasis is given to the immune-chemical methods, which are described in detail. The next section is a short description of the many subfractions of the serum proteins and their special function and their separat turn-over rates. The greater part of the book is devoted to the clinical significance of the different proteins and their pathology This section has been written together with four collaborators, and a wealth of knowledge has been collected. The literature references make up almost one quarter of the book, and many of the tables and figures are from the thor own publications. New entities such as the auto-immune disease and the ant body deficiency syndrome are extensively described. The new syndromes of an albuminuria and beta-lipoprotein deficiency have also been included. The latter includes

besides lack of the beta lipoproteinaemia, torrhea, dwarfism, retinitis pigmentosa and a neurological disorder with ataxia. Finally in the last chapter a brief summary is given of the interesting genetics involved in the study of the distribution of the different subgroups of plasma proteins such as haptoglobulins and transferrin. The authors have succeeded in giving a well balanced presentation of our present-day knowledge. Not all is easy reading and many problems are still unsolved. The reviewer would have liked to get more information about special problems as for example hemophilia, organ specific autoimmune antibodies, and the fluorescence antibody reaction, but the book can be highly recommended for any doctor who wants to get a good background knowledge of these very important subjects. Furthermore it is equally handy as a reference book for quick orientation, which is facilitated by making all the pages containing literature references black in the upper corner.

Bent Friis Hansen, Copenhagen

E. Rossi (ed.) Postgraduate Courses in Pediatrics, Kinderpsychiatrie in der Praxis No. 9

S. Karger, Basel (Schweiz) and New York, 1963. Price DM 1..

Seven papers by different authors offer short concentrated and perfect survey of the main chapters in child psychiatry. It is, however extraordinary how much the trend in the German-speaking countries are influenced by the classical psychiatry of adult. Stutte writes an excellent paper with very representative case histories concerning child psychoses but he only quotes two American writers, one of them a psychiatrist for adult in spite of the fact that the Anglo-Saxon countries have a very rich choice of good papers on this subject. Sauter makes some very important point in her chapter about the function of a psychiatrist in children's hospital, how important it is to underline the fact that children with symptoms of organic lesions can improve by changement of environment exactly as well as children with symptoms of psychogenetic disease. The book is easy to read, certainly nothing new for a child psychiatrist but gives good child psychiatric orientation for the pediatrician which is the declared purpose of the book.

Elsa Britt Nordlund, Stockholm

ANNOUNCEMENTS

Congresses of Pediatrics

The 11th International Congress of Pediatrics will be held in Tokyo November 7–13, 1965.

The IVth Middle Eastern Mediterranean Pediatric Congress, Athens Sept. 27–30, 1964. Professor C. Choremis is president and

Address: 79 Stadium Street, Athens, Greece. The programme will include papers on subjects related to Hematology, Endocrinology, Metabolic and Nutritional Diseases, Neonatal Physiology and Pathology, Infectious and Parasitic Diseases as well as papers of

Acta Paediatrica 53 401–407 Sept 1964

From the Department of Pediatrics, Harvard Medical School and the Department of Medicine Children s Hospital Medical Center Boston

Pulmonary Function in Symptom Free Asthmatic Patient.

by MARCELLO M. ORZALESI, CHARLES D COOK and MONTGOMERY C. HART

Previous studies of pulmonary function in patients with asthma have resulted in conflicting conclusions concerning the amount of residual respiratory abnormality during symptom free periods [1 2, 9 10 11 20 1 22, 26 27]. The present investigation provides further information concerning the pulmonary function of asthmatic children during asymptomatic periods and of adults who had asthma during childhood and were also asymptomatic at the time of study

Material and Methods

The 29 patients studied were divided into tw groups. Nineteen children (12 boys and 7 girls) between 7½ and 15½ years of age, comprised the first group; they had had asthma for at least three years with four or more severe attacks per year and had required intensive therapy and admission to the hospital on numerous occasions. The second group consisted of 10 adults (8 men and 2 women) between 17 and 34 years of age who had had severe asthma requiring frequent admissions to the hospital during childhood. Only two of these adult still exh bited occasional mild symptoms of asthma and received intermittent treatment with bronchodilators. At the time of study none of the patient in either group was taking any medication or had any ymptoms or signs of asthma. None of the patients had a hronic cough or any other signs of hronic pulmonary infection.

The following measurements were made: ( ) lung volumes, using the closed-circuit helium dilution technique described by Hellsesn et al. [5, 19]; (b) anatomic dead space (V_D) by the single breath nitrogen wash-out method of Fowler [12 15], ( ) airway resistance t FRC using with minor modifications, the technique of Dubois et al [8, 16]; (d) peak expiratory flow rates (PFR) using the Wright peak flow meter [23, 28]; ( ) one and two-second timed vital capacity ($TVC_{1 sec}$ and $TVC_{2 sec}$) using the technique described by Gaensler [14, 25]; (f) maximum breathing capacity (MBC) using a one-way valve and a Douglas bag [24 25], (g) distribu tion of inspired gas as described by Briscoe et al. [4 17]; (h) maximal st tic expiratory and inspiratory airway pressures t different lung volumes as described by Cook et al. [6]. The results of each test were compared with normal values obtained by the same techni ques in the same laboratory

Results

The results of the measurement of lung volumes, expressed as per cent of the predicted value for both groups of patients are shown in Table 1 and Fig. 1 In the first group of patients the mean residual *volume* (RV) *was higher than predicted* ($P < 0.025$) but only 3 of the 19 children

Supported in part by Grants No. HD-00248-04 and 2A-5276 from the National Institutes of Health.

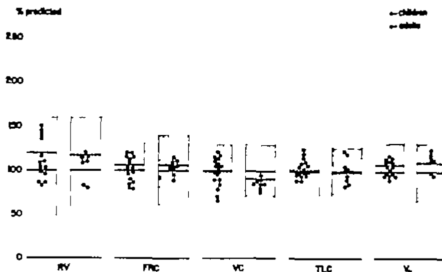


Fig. 1 Average (——) and individual lung volumes in asthmatic children (●) and adults (○). The values are expressed as per cent predicted on the basis of height; the continuou line and the shaded areas represent the verage ±2 S.D for the normals [12, 14].

had a significant increase in RV exceeding 2 standard deviations. The mean values for all the other lung volumes in this group were within normal limits. When considering individual values, one patient had a significant increase in functional residual capacity (FRC) 2 had decreased vital capacities (VC) and one an increased total lung capacity (TLC) (Fig 1)

The mean values for all the lung volumes

1 *Pulmonary function tests in asthmatic children and adults during symptom free periods*

Pulmonary function tests[a]	Normal		Children			Adult		
	Average	S.D	Average	S.D	P	Average	S.D.	P
RV	100	30	120	36	< 0.025	118	39	N.S.
FRC	100	20	106	22	N.S.	106	20	N.S.
VC	100	15	100	19	N.S.	91	1	N.S.
TLC	100	14	10?	16	N.S.	10?	13	N.S.
V_D	100	17	108	22	N.S	110	13	N.S.
C_L at FRC	100	27	61	31	< 0 025	77	32	0 025
PFR	100	15	107	23	N.S.	100	11	N.S.
MBC	100	20	7	I	< 0.001	79	11	< 0.001
Air velocity index	0.909	0 163	0.758	0.175	< 0 001	0.901	0 154	N.S.
TVC sec children	83.5	6.0	73.9	9 4	< 0.001	—		—
TVC sec adults	76.6	6.2	—	—	—	1 7	7 4	N.S.
TVC sec children	94.5	3.5	89.3	6.5	0 005	—	—	—
TVC sec adults	90.7	5.6	—	—	—	90.2	6 5	N.S.
N wash-out delay	13.4	10.9	32.1	23.9	< 0.005	?8.1	33 6	N.S.

The result of the test are expressed as per cent of predicted on the basis of height except for air velocity index, TVC sec and TVC sec and nitrogen washout delay

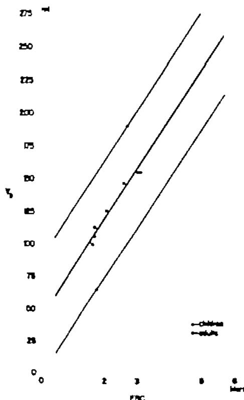


Fig. 2. Anatomic dead space (V_D) versus functional residual capacity (FRC) in asthmatic children (●) and adults (○). The regression line ±2 S.D. for normal subjects is also shown [14].

in the group of adults were within normal limits. Only one patient had a significantly increased (>2 S.D.) RV and the same patient also had an increased FRC (Fig 1)

The mean anatomical dead space (V) for each group of patients wa normal when the predictions were made on the basis of either height or FRC (T ble 1 Fig. 1 and) Only patients of the first group had an increased V (>2 S.D.) when compared with normal on the basis of height, but all were within normal limit on the basis of their FRC

The mean values for airway conductance (C_a) the reciprocal of airw y resistance in both groups of patient were slightly but significantly ($P = <0.0$) decreased when compared with normal values on the basis of height (Table 1) or FRC (Fig 3)

The mean values for TVC$_{1\,sec}$ and TVC$_{\,sec}$ were significantly lower than predicted only in the group of children ($P<0.001$ and $P<0.005$) Mean values for MBC were also decreased ($P<0.001$ for both groups of patients) but only 4 children and one adult had significantly (> S.D.) low values. On the other hand, another indi rect method for estimating airway obstruction, the measurement of peak flow rates, showed no significant variation from normal for either group or for the indi vidual (Table 1).

The air velocity index, the ratio of the per cent predicted MBC to the per cent predicted VC has been used by Gaensler [13] to demonstrate abnormal pulmonary function in asthmatic patients In the

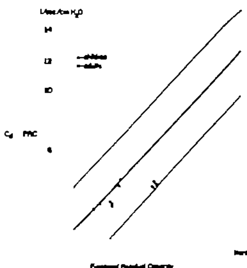


Fig 3. Airway conductance (C_a) versus functional residual capacity (FRC) in asthmatic children (●) and adults (○). The regression line ±2 S.D. for normal subjects is also shown [16].

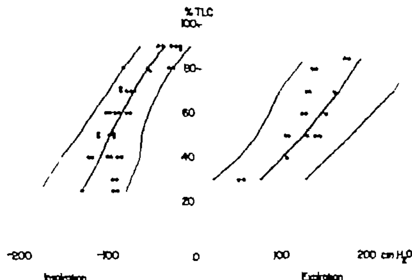


Fig. 4. Maximal inspiratory and expiratory airway pressures (cm H_2O) at different lung volumes (%TLC) in 8 asthmatic children. The average values ±2 s.d. from 14 normal children are also shown [6].

present series it was significantly reduced for the children ($P < 0.001$) but not for the adults.

The mean nitrogen washout delay was significantly increased in the children ($P < 0.005$) but within normal limits in the adults (Table 1). Eight children and 2 adults had an abnormally increased (>2 s.d.) delay.

The maximal airway pressures on expiration and inspiration at different lung volumes were determined in 8 patients of the first group. The results were compared with those of a group of 14 children of approximately the same age and height (Fig. 4). Only one patient of the 8 had low inspiratory and expiratory pressures.

Discussion

The principal cause of pulmonary dysfunction in asthma is bronchial obstruction which results in a reduction in gas flow especially on expiration, and secondary air trapping and hyperinflation. It has been suggested that repeated attacks of asthma might lead to rupture of the alveoli and loss of elastic tissue with subsequent irreversible emphysema.

Direct measurements and indirect estimations (PFR, TVC and MBC) of airway resistance, the distribution of inspired gas (which is abnormal when partial obstruction is present) and the measurement of lung volumes, especially RV, should be the most useful tests for evaluating the presence or absence and extent of pulmonary changes due to asthma.

A number of authors have studied both asthmatic children and adults during symptom free periods with variable results. Lukas [22] found that increases in the RV/TLC ratio were apparently not reversible in two of 6 asthmatic children tested during symptom free periods. He postulated that severe asthma might fre-

quently result in chronic emphysema. Andrewes & Simmons [1] also found changes in lung volumes (an increase in RV and FRC) in 21 symptom free asthmatics. Beal [ ] studied 20 patients ranging in age from 11 to 66 years and found slight hyperinflation (increases in RV and FRC) a decrease in MBC and abnormalities in intrapulmonary gas distribution. These changes were partly or wholly reversed following the administration of bronchodilators. Tooley reported similar findings [27]. Kraepelien & Engström and co-workers [11 21] have shown that children with asthma have abnormalities in pulmonary function during asymptomatic periods; these changes decreased with age and reached normal values in most of their patients. Consistent with the data of Kraepelien are those of Sadoul *et al.* [26] who reported that 74% of 128 young men with asthma had normal lung volumes and TVC during symptom-free periods.

The present study indicates that the measurement of residual volume airway resistance MBC, TVC air velocity index and nitrogen washout delay are the most sensitive tests for demonstrating changes in asymptomatic asthmatic patients. These parameters are significantly although slightly abnormal for groups of asymptomatic asthmatic patients but only a few individual patients have values which vary significantly from the normal. Thus although pulmonary changes persist in a few children and adults during symptom-free periods, they are minimal. Unfortunately in the present series the further reversibility of the minimal changes after the administration of bronchodilator drugs was not investigated.

Engström & Karlberg [11] using a different technique, did not find a significant increase in total flow resistance. The Dubois technique used by us measures only air flow resistance rather than total pulmonary resistance and may be more sensitive in detecting changes due to obstruction. Furthermore, since air flow resistance decreases with increased lung volume [8 16] it may be that their measurements of airway resistance would have been elevated if they had been compared to normal values on the basis of FRC rather than height. Another possible explanation for the discrepancies might be a difference in the severity of the asthma, it seems unlikely however that our patients had more severe disease in view of the minimal changes in lung volumes and the strict criteria for selecting asymptomatic asthmatic patients without complications.

The Wright peak flow meter has proved satisfactory for following obstructive changes in symptomatic asthmatic patients both in our laboratory [23] and in the reported work of others [18]. However it is apparent that this test was not as sensitive for detecting minimal changes in asymptomatic patients as the $TVC_{1\ sec}$ and $TVC_{3\ sec}$, the MBC, the air velocity index or the direct measurement of airway resistance. Consistent with the finding of an increased airway resistance were the results of the nitrogen washout test which showed uneven gas distribution in about a third of the patients.

The anatomic dead space is not apparently affected by recurrent asthma as no significant increases were noted. This suggests that there was not enough change in the structure of the air passages to result in detectable changes in this volume.

It was considered possible that recurrent attacks of asthma might be associated with hypertrophy of the respiratory muscles, particularly those used on expiration but no such change was found. This is consistent with earlier findings in severely emphysematous elderly men who showed no evidence of increased expiratory muscle strength when compared to normal persons of the same age [7]

In conclusion, the present data and a review of the literature indicate that the majority of asthmatic patients have pulmonary function which is within normal limits during symptom free periods. However if they are considered as a group instead of individually they may show some slight abnormalities, particularly in their airway resistance indicating a minimal and subclinical airway obstruction.

Summary

Evaluation of pulmonary function in 29 [illegible]umatic patients (19 children and 10 [illegible]lults) during symptom free periods gave the following results.

1 Twenty five of the 29 patients had normal residual volumes, 27 had normal functional residual capacities, and 28 had normal vital capacities. Both groups of patients showed a moderate increase in mean residual volume (significant only in children) all the other mean lung volumes being normal.

2. The mean anatomical dead space for the two groups of patients was normal on the basis of both height and functional residual capacity

3 The mean airway resistance was significantly increased in both groups of patients and the maximal breathing capacity was consistent with this finding i e lower than expected. The timed vital capacity (one and two second) and the air velocity index were significantly low only in the group of children. Peak expiratory flow was normal in both groups, indicating that the small increase in airway resistance was not detectable by this technique

4 The nitrogen washout test showed uneven distribution of inspired gas in 10 patients (8 children and [illegible] adults) and was normal in 18

5 Respiratory muscle strength was measured in 8 children and was normal in 7

6 These results indicate that complete or almost complete regression of the changes in lung function is to be expected in the majority of patients with asthma during symptom free periods If the patients are considered as a group however they show a small but significant increase in airway resistance

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Conduction Velocity of Peripheral Motor Nerves in Mental Retardation, Diabetes and Various Neurological Diseases in Childhood

by INGRID GAMSTORP

In a previous publication the normal values were given for conduction velocity of peripheral motor nerves in infancy childhood and adolescence [3] The values found for the ulnar median and peroneal nerves in different age-groups were interpreted as an expression of the normal maturation of the peripheral nervous system It was therefore considered of interest to assess the conduction velocity of peripheral nerves in groups of sick children with either a conceivably slow maturation diseases capable of affecting the peripheral nerves. Three groups were studied. 1 Mentally retarded children. 2. Diabetic children. 3 Children with neurological diseases, possibly affecting the peripheral nerves All the patients were below 16 years of age

Methods

The method described by Hodes, Larrabee & German [6] was used with small modifications previously reported in detail [3]. The method, as applied to the ulnar nerve is schematically outlined in Fig 1 A supramaximal electrical stimulus was given percutaneously at different points along the nerve and the evoked response of a peripheral muscle was picked up by surface electrodes and recorded on a Disa electromyograph. The conduction velocity of the nerve itself was calculated by dividing the difference between the 2 recorded conduction times by the distance measured on the arm between the point of stimulation. The ulnar median and peroneal nerves were routinely examined. The ulnar nerve was also examined for the presence of Hoffmann reflex (H reflex) [12].

I Mental Retardation

Material

All together 58 nerves in 47 patients, 28 boys and 19 girls, were examined. Fourteen children were between 1 and 3 years of age 16 between 3 and 8 and 17 between 8 and 16. Infants were not included because of the difficulty in evaluating the intellectual development in this age-group. All children were severely mentally retarded. The main part of the older children were inpatient of the St t Hospital in Lund for the Uneducable (Vipeholms Sjukhus). Twelve children were mongols, 2 in the oldest and 5 in each of the

Part 3 of this material belongs to study supported by grant from "Solstickan Foundation through the Swedish Medical Research Council.

TABLE 1 *Conduction velocity of peripheral nerves in groups of children with mental retardation (B) and with diabetes (C) and in age matched controls (A)*

	Ulnar nerve		Median nerve		Peroneal nerve	
Age-group	No. of observations	Mean	No. of observations	Mean	No. of observations	Mean
			A. *Normal material*			
1-3 years	21	59.8 ± 8.1	1	49.5 ± 5.9	21	53.7 ± 8.1
3-8 years	26	65.4 ± 8.5	[illegible]	58.3 ± 5.4	26	57.5 ± 6.9
8-16 years	26	67.8 ± 6.0	20	63.9 ± 5.7	20	57.8 ± 7.2
			B. *Mental retardation*			
1-3 years	26	62.9 ± 11.2	26	4[illegible]8 ± 8.5	26	51.7 ± 9.1
3-8 years	27	58.8 ± 6.6	7	50.1 ± 7.4	27	54.7 ± 5.9
8-16 years	33	63.2 ± 8.0	33	57.2 ± 6.9	33	52.7 ± 5.3
			C. *Diabetes*			
3-8 years	16	59.1 ± 6.2	16	58.6 ± 9.5	15	49.2 ± 6.5
8-16 years	30	59.9 ± 6.1	29	58.0 ± 4.3	30	5[illegible]0 ± 6.0
			A-B			
1-3 years	−2.1	$p > 0.03$	6.7	$0.01 > p > 0.001$	2.0	$p > 0.05$
3-8 years	6.8	$0.01 < p > 0.001$	8.[illegible]	$p < 0.001$	2.8	$p > 0.05$
8-16 years	5.4	$0.01 > p > 0.001$	6.6	$p < 0.001$	4.9	$0.03 > p > 0.01$
			A-C			
3-8 years	6.3	$0.01 > p > 0.001$	−0.3	$p > 0.05$	8.2	$p < 0.001$
8-16 years	7.7	$p < 0.001$	8.9	$p < 0.001$	5.6	$0.01 > p > 0.001$

younger age-groups. Fou had a history of organic brain damage in the neonatal period one had tuberous sclerosis, and 2 had untreated phenylpyruvic oligophrenia. In the remaining 28 the cause of the mental retardation was unknown. Patients with symptoms of a progressive disease of the central nervous system were excluded, as were diabetics and children with symptoms possibly referable t   disease of the peripheral nervous system such as muscular atrophy or loss of deep tendon reflexes. The aim was to include only children with early onset of symptoms; in all the children studied retardation of psychomotor development had been observed within the first year of lif

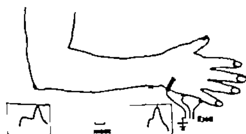


Fig. 1 Schematic presentation of the method applied t   he ulnar nerv

Results

Of the [illegible]58 individual measurements 9 (in the ulnar nerve 5 in the median nerve and 2 in the peroneal nerve) were considered definitely below the normal limit for age. Borderline low values were found in 4 ulnar in 14 median and in 8 peroneal nerves. The means for the 3 different nerves in the 3 age-groups were calculated and compared with the mean for

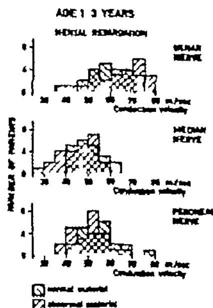


Fig —. Conduction velocity of peripheral nerves in pparently healthy children and mentally retarded children, 1 3 years of age

apparently healthy age matched children [3] Four of the 9 means did not differ significantly from the normal means (the peroneal nerve in all age-groups and the ulnar nerve in the youngest age-group), 3 (the ulnar nerve in the 2 older age-groups and the median nerve in the youngest age-group) were significantly below the normal means ($0.01 > p > 0.001$) and in (the median nerve in the 2 older age-groups) the difference was highly significant ($p < 0.001$) Thus the group of mentally retarded children appeared to have a lower conduction velocity in the nerves of the arm at least and particularly in the median nerve whereas no deviation from the normal values was apparent in the leg. The results are summarized and compared with normal values in Table 1 and in Fig 2, 3 and 4

The H reflex was absent in all the ulnar nerves examined.

Discussion

The changes in conduction velocity of peripheral nerves through normal infancy childhood and adolescence are considered a reflexion of the functional maturation

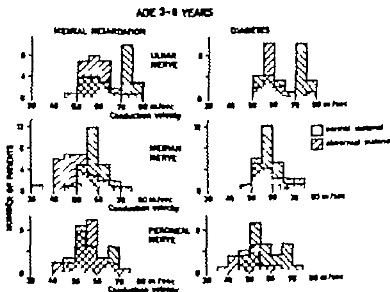


Fig. 2. Conduction elocit of peripheral nerves in pparently health children, mentally retarded (left) and diabetic children (righ), 3–8 ears of age

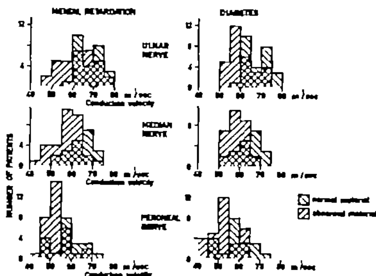


Fig. 4. Conduction elocity of peripheral nerves in pparently healthy children, mentally retarded children (left) and diabetic children (right), 8–16 years of age

of the peripheral nervous system [3 18] The findings set forth above therefore sug gest a retarded development at least of the median nerve in the group of mentally retarded children. In normal children the maturation of the nerves of the arm, and particularly of the median nerve lasts longer than that of the peroneal nerve which at age one conducts with practically the same velocity as in normal adults [3]. Most mentally retarded children learn to run and all children above 3 in the present material were able to walk by them selves. Their ability to perform small, exact finger movements is, however often severely retarded and they seldom achieve normal adult skill, particularly regarding the precise movements of the thumb It is tempting to assume a connexion between the clumsy finger movements of the mentally retarded children and the delayed functional maturation of the peripheral nerves of their arms. The interpretation of the results in the mentally retarded children is further discussed in relation to the results obtained in diabetic children (see next page)

It should be stressed that the difference between normal children and mentally retarded children was demonstrable only when the 2 groups were compared with each other *as gro ps* Practically all the *individual value* fell within normal limits Measurement of the conduction velocity of the peripheral nerves is thus of no help in the evaluation of the mental development of given person.

No lesion or slow maturation of the pyramidal tract could be demonstrated by the method used, i.e. examination of the ulnar nerve for the H reflex in children bove one year

II Diabetes

Material

The material consisted of 23 diabetic children (15 boys and 8 girls) in whom 136 nerves were examined. Eight of the children were between 3 and 8 years of age and 15 between 8 and 16. The duration of the disease varied between 0 and 10 years. At the time of examination all the children were under treatment and free of ketoacidosis. Deep tendon reflexes were normal. No case of obvious mental retardation was included.

Results

Of the 136 individual measurements one (peroneal nerve) was considered definitely below the normal limit for age and 8 (one median nerve and 7 peroneal nerves) were borderline low. A mean was calculated for each nerve and each age-group and compared with the normal means [3]. No difference was found for the median nerve in the younger age-group. The values for the ulnar nerve in the younger and for the peroneal nerve in the older group were significantly decreased ($0.01 > p > 0.001$); the difference was highly significant for the peroneal nerve in the younger and for the ulnar and median nerves in the older age-group ($p < 0.001$). The results are summarized and compared with normal values in Table 1 and Figs. 3 and 4.

No H reflex was found in the ulnar nerve.

Discussion

The high incidence of clinical signs of polyneuropathy in diabetes has prompted several investigations on the motor nerve conduction velocity in this disease. The results are concordant: a higher proportion of abnormally low values and lower means in diabetics with or without clinical signs of polyneuropathy than in apparently healthy persons [2, 14, 15, 1]. The published materials consist mainly of adults: the youngest patient of Mayer [14] was 10, of Skillman *et al.* [1] was 1., and 4 of the 103 patients of Mulder *et al.* [15] were between 11 and 19. The number of individual low values appears smaller in the present childhood material than in adult materials. This holds true when the present material, in an attempt to eliminate the possible effect of the duration of the disease, is compared with those patients, reported by Mulder *et al.* [15], who had had diabetes for at most one year. Of 34 patients in this group, 4 had certain mononeuropathy and 6 polyneuropathy, whereas among the present material of 23 children no case of polyneuropathy and only one of mononeuropathy were found, although several of the children had had diabetes for more than one year. However, mean values for diabetic children, as well as for diabetic adults, are significantly lower than for age-matched controls and thereby suggest some functional disorder of the peripheral nervous system also in young diabetics.

The results in diabetic children were compared with those in the mentally retarded group. In the diabetic group, in which a disease of peripheral nerves is postulated, the mean values were lower than normal both in the arms and in the legs and if anything more consistently so in legs. In the mentally retarded, on the other hand, in which a slow maturation is postulated, the mean values were abnormally low only for the nerves of the arm and particularly for the median nerve.

TABLE 2. *Conduction velocity of peripheral nerves Results of measurements children with various neurological diseases*

Diagnosis	No. of patients examined	No. of patients with abnormal findings	No. of patient with borderline values
Late-infantile metachromatic leucodystrophy	1	1	0
Diffuse brain sclerosis, not classified	3	0	0
Acute polyneuropathy	2	2	0
Primary muscle diseases			
Myositis	2	0	0
Progressive dystrophy	7	0	0
Myotonic dystrophy	1	0	0
Not classified	3	0	1
Spinal muscular atrophy			
Polio	1	0	0
Progressive infantile	2	0	1
Progressive juvenile	3	0	0
Chronic polyneuropathy	11	10	1

III. *Neurological Diseases*

Material and results are summarized in T ble 2

Discussion

The first case was in a * year-old boy with the onset of pyramidal tract symptoms at the age of 11 months. The disease progressed steadily and the patient had at the time of xamination severe pyra midal tract symptoms ataxia, severe mental deterioration and tonic fits. The diagnosis was established by the finding of a high protein concentration with a normal relative distribution of the protein fractions in the spinal fluid and the pathognomonic finding on urinary paper chromatography of one large metachromatic sulphatid spot and a second more slowly running spot with the same colour [4] This boy had low conduction velocity of one nerve and re examination month later showed progress with bnormal values in 3 nerves. Involvement of the peripheral nervous system is often seen in this disease It manifests itself histologically as a brown metachromatic deposit in nerve sheaths [6] and clinically as signs simulating poly radiculitis [5]. Although the boy showed no clinical signs of polyneuropathy Im paired function was demonstrated by the measurement of the conduction velocity In 3 other patients with clinical signs of a progressive disease of the central nervous system but definitely not metachromatic leucodystrophy the conduction velocity was found to be normal.

Two patients with acute polyneuropathy are included in the material both had abnormally low conduction velocity One had a classical type of Guillain-Barré syndrome the other cute encephalomyeloneuritis whi h had ppeared fter an exanthematous disease probably rubella Peripheral nerve function re-

covered completely in both, clinical im provement started several months before conduction velocity became normal. This is in agreement with observations made in larger series [11 16].

The rest of the listed patients had difficulties to move about and symptoms pointing to a disease of the motor unit. The clinical examination was supplemented by determination of the serumenzymes electromyography and histological examination of a muscle biopsy specimen, and the diagnosis was based on the results of these examinations. The conduction velocity was normal in all cases of a primary muscle disease except one The exception was a 6-month-old boy who since birth had a severe primary muscle disease of undecided type this boy had borderline values in 2 of 6 nerves examined. The reason for this finding remains uncertain, no other methods could be used in this young infant to exclude or prove the possible presence of peripheral neuropathy

Patients with a myelopathy also had a normal conduction velocity with the exception of one infant with progressive spinal muscular atrophy who showed borderline values in 2 of 6 nerves examined A possible explanation for this finding is that the chronic progressive disorders of the lower motor neuron usually affect the large anterior horn cells first and most severely [19] The thickest and fastest conducting nerve fibres belong to the largest anterior horn cells; as the method used allows measurement only of the fastest conducting fibres this will mean an unmasking of normal thinner fibres and a slight decrease of the conduction velocity

The results in patients with primary muscle diseases or myelopathy contrast with the findings in the next group the 11 children with probable chronic polyneuropathy 10 of whom had a definitely abnormal conduction velocity in at least 2 of the 6 nerves examined. This group included twin brothers with Friedreich ataxia Six children had abnormally slow conduction velocity in all the nerves examined None in the group showed completely normal values one girl had borderline values in 2 nerves.

The examination of the conduction velocity of peripheral nerves has been well established as a valuable diagnostic tool in distinguishing between different neuromuscular disorders [7 9 10 11 13] and the presented results are in agreement with previously reported series. The difference is usually large enough to be diagnostic between the mild reduction of conduction velocity occasionally found in diseases of the anterior horn cells and the low velocity noted in cases of chronic polyneuropathy [11] The occurrence of this disease in childhood and the diagnostic difficulties in this age were stressed by Byers & Taft [1] They emphasized the importance of a correct diagnosis, a chronic polyneuropathy carries a better prognosis than do the diseases for which it is easily mistaken, i e progressive muscular dystrophy and progressive infantile spinal atrophy

Lambert [11] stressed the value of measuring conduction velocity of peripheral nerves in infants and children, in whom a good sensory examination is often difficult to perform and to interpret As the method is simple and inconveniences the patient only slightly it definitely belongs to the diagnostic tools of pediatric neurology

Summary

The conduction velocity of ulnar median and peroneal nerves was examined in 3 groups of sick children. 1 Mentally retarded children. 2. Diabetics. 3 Children with various neurological diseases, possibly affecting the peripheral nerves.

In the first group few individual values fell outside normal limits for age The mean was calculated for each nerve and compared with the mean for normal children of the same age For the nerves of the arm, particularly for the median nerve the nerve which is normally the latest to reach normal adult level, the mean was significantly below normal, whereas the mean for the peroneal nerve did not differ from normal. This may be interpreted as a sign of delayed maturation of the peripheral nervous system, a possible connexion is discussed with the clumsy finger movements, particularly of the thumb in mentally retarded children.

In the second group few individual values fell outside the normal range the means were significantly below normal in the arms and in the legs. Thus, in diabetic children in whom a disease of the peripheral nerves appears the likely explanation for the decreased conduction velocity all examined nerves were affected and, if anything the peroneal nerve more so than the nerves of the arm

In the third group individual values for conduction velocity falling definitely below the normal limit were found in disorders affecting the peripheral nerves, e.g late-infantile metachromatic leucodystrophy acute and chronic polyneuropathy In diseases affecting exclusively the central nervous system or the muscles the results are normal or occasionally low borderline The method has its greatest value in children in whom it is difficult to interpret the results of other neurological examinations particularly of sensation.

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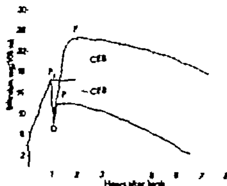


Fig. 1 The course of bilirubin concentrations in infants with haemolytic disease of the newborn.

Results

A. *Factors associated with the production of bilirubin*

Since the rate of production of bilirubin is directly related to the rate of haemolysis it may be predicted that the rate of its production after exchange transfusion would be proportional to the quantity of residual haemoglobin in nonexchanged foetal erythrocytes. The relative and absolute amount of this vulnerable haemoglobin can be calculated from the fall in the proportion of alkali resistant haemoglobin occurring during exchange transfusion (6)

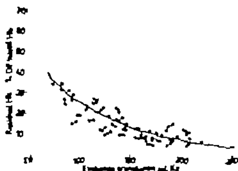


Fig. — The relationship between the amount of blood exchanged and the fall in original haemoglobin in Cases with atypically low exchange (probably due to technical failure)



Fig 2. The relationship between the amount of blood exchanged and the amount of residual haemoglobin in g/100 ml. ● Cases with mild or moderate anaemia; ○ cases with severe anaemia (Hb < 10 g/100 ml) Atypical exchange (see Fig ?).

Fig 2 shows the amount of blood exchanged in ml/kg body weight plotted against the per cent of foetal haemoglobin remaining after exchange transfusion The regression curve of this relationship is asymptotic, its equation being given by $y = 10^{2-0.004x}$

Fig 3 shows that approximately the same relationship is found if the absolute amount of residual haemoglobin is plotted against the amount of blood exchanged. The scatter of values is greater however because of the variability of pre-exchange haemoglobin levels. Severely anaemic infants (open circles) tend to have lower amounts of residual haemoglobin for a given size of exchange transfusion With exchanges over 160 ml/kg virtually all infants had residual haemoglobin below 2 g/100 ml

In Fig 4 values of residual haemoglobin are plotted against CEB in full term infants with HDN treated within 48 hours of birth. In infants with Rh incompatibility (solid circles) the post-exchange accumu

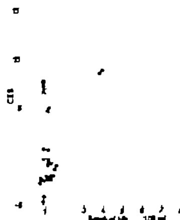


Fig. 4. Residual haemoglobin and CEB (clinically excess bilirubin) in term infants, treated within 48 hours of birth. ● Rh incompatibility; ○ ABO incompatibility; ◯ repeated exchange transfusion; *K* kernicterus; † deaths.

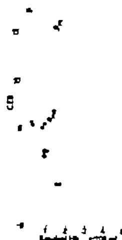


Fig. 5. Residual haemoglobin and CEB in premature infants (symbols as in Fig. 4).

lation of bilirubin always occurred when the amount of residual haemoglobin was more than about 2 g/100 ml, when the residual haemoglobin was below this limit CEB was negative in 70 % of cases. The difference between infants with low and high residuum of haemoglobin is significant ($\chi = 12.67610$ $P < 0.01$). Infants with HDN on the basis of ABO incompatibility (open circles) seemed to tolerate greater amounts of vulnerable haemoglobin.

The upper values of CEB were not found to be related to the size of the haemoglobin residua, and large accumulation of bilirubin after exchange transfusion occasionally took place with very effective elimination of foetal cells as judged by residual haemoglobin.

In premature infants (Fig. 5) values of CEB were almost always positive regardless of the quantity of residual haemoglobin.

No relationship could be found between CEB and the severity of haemolytic disease present, as judged by the titer of maternal antibodies or the rate of rise of bilirubin concentration in mg/hour during the first day of lif

B. *Factors associated with the excretion of bilirubin*

1. *The degree of gestational maturity.* The columns of Fig. 6 indicate weeks of gestation, determined obstetrically as accurately as possible. Only infants with Rh incompatibility treated within 48 hours of birth, are included.

Infants born at term (39–41 weeks) showed negative values of CEB in the majority of cases. In contrast 90 of infants of 38 or less weeks gestation accumulated excess bilirubin following exchange transfusion. This difference is significant ($\chi = 11.0176$ $P < 0.01$).

Haemolytic disease on the basis of ABO incompatibility was so rare in pre-

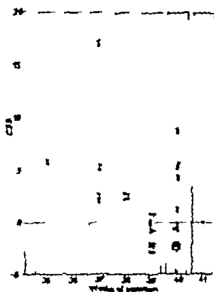


Fig. 6. Duration of gestation and CEB (only infant with Rh incompatibility treated within 48 hours of birth).

mature infants that no comparison could be made

2 *Postnatal age* Fig 7 shows values of CEB plotted against the age of infants at the start of exchange transfusion in full term infants.

The time at which exchange was carried out markedly affected the post exchange accumulation of bilirubin with the highest values of CEB found in infant treated within 1 hours of birth Thereafter upper values of CEB decreased with increasing postnatal age and from the beginning of the fourth day only negative values of CEB were found On the average cases with ABO incompatibility were treated later than those with Rh incompatibility and their values tend to be lower

In most premature infant (Fig 8) exchange was carried out soon after birth and it was not possible to assess the influence of postnatal age

Discussion

There are two mechanisms by which bilirubin concentrations in HDN can be prevented from rising above critical level

1 Direct removal of previously formed bilirubin causes a 5 to 30 reduction in plasma bilirubin levels [4] It has been shown that the absolute amount of bili-

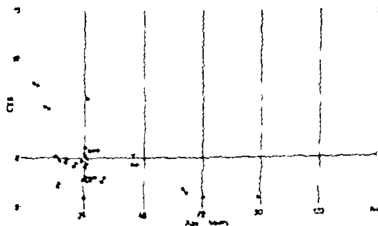


Fig 7 Postnatal age at the start of exchange transfusion and CEB in term infants ● Rh incompatibility; ○ ABO incompatibility

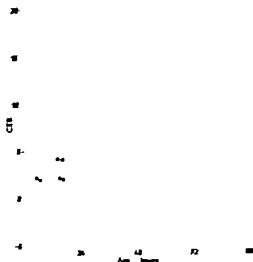


Fig. 8. Postnatal age at the start of exchange transfusion and CEB in premature infants (symbols as in Fig. 7).

rubin removed increases continually though not in linear fashion, with the amount of blood exchanged [2, 5].

2. Removal of the sensitized red cells and antibodies prevents continuance of abnormally high rates of bilirubin production. The reduction in numbers of remaining foetal erythrocytes, however decreases exponentially as the size of exchange transfusion increases, and thus the efficiency of removal of the haemolytic system falls considerably during the course of the procedure

Prevention of continuing haemolysis plays a far more important role quantitatively- one can easily calculate that removal of 80–90 % sensitized red cells prevents the formation of an amount of bilirubin many times that ctually removed by exchange transfusion.

Our data show that the degree of gestational maturity of treated infant is apparently the most important factor influencing effectiveness of exchange transfusion. Almost all premature infants showed positive often very high, values of post-exchange accumulation of newly formed bilirubin, whatever the amount of residual haemoglobin (Fig 5). Exchange transfusion had to be repeated in 25 %, as compared to 10 % in full term infants.

In full-term infants, a rise in bilirubin concentration could be entirely suppressed by a single exchange transfusion when the residual haemoglobin was less than about 2 g/100 ml This was virtually always achieved by an exchange of 160–1 0 ml/kg often by less (Fig 3). Even in this group with low residual haemoglobin, in about a third of the cases, post-exchange accumulation rose as high as in infants with a much less effective exchange as judged by the residual haemoglobin (Fig. 4) Such cases probably represent individually low excretory capacities since this function shows considerable individual variation.

These conclusions are fully valid only for newborn infant treated within 48 hours of birth. This is of course the most common situation. In infants treated later the distinct though poorly understood improvement in post-natal excretion of bilirubin by the liver causes a rapid decrease in the post-exchange accumulation of bilirubin, and from the beginning of the fourth day the exchange was always successful" i.e. with negative CEB (Fig 7 and 8) This is, however of little practical importance because the decrease of CEB is cancelled out by the higher saturation of the body by bilirubin thus the peak levels after transfusion are about the same in infants treated early as later

It may be concluded that when an adequate amount of blood, i 150 ml/kg

or more is exchanged (Fig 3) the failure of the first exchange transfusion, often necessitating repeated exchange is due mainly to inadequacy of bilirubin excretion, secondary to gestational immaturity or individual inefficiency of hepatic conjugation. These endogenous factors are little influenced by external efforts and thus little can be achieved by prolonging the procedure No difference in results could be demonstrated when the amount of blood exchanged varied from 150 to 250 ml/kg We therefore give an average of 150 ml/kg in about 90 minutes This amount may be decreased to 100–130 ml/kg in mild cases particularly with ABO incompatibility and in all cases treated after 72 hours.

In premature infants with a high level of bilirubin (20 mg/100 ml or more) the size of the exchange may be increased from 150 ml/kg to 200–250 ml/kg in order to reduce body pools of bilirubin as much as possible We feel however that in such cases repeated exchange transfusions when necessary are both safer and more effective

Summary

The difference between the highest level of bilirubin concentration before and after exchange transfusion has been used a s measurable index of the effectiveness of exchange transfusion.

During the first two days of life exchange transfusion was entirely successful only when the residuum of haemoglobin in foetal cells did not exceed about g/100 ml. This was virtually always achieved by an exchange of 150–170 ml/kg, often by smaller quantities. Under these conditions the failure of exchange transfusion was chiefly due to inadequacy of bilirubin excretion secondary to gestational immaturity or individual variability In our data the outcome of exchange transfusion was not dependent upon the size of the exchange (150–250 ml/kg)

We give approximately 150 ml/kg of blood in about 90 minutes This amount may be decreased to 100–130 ml/kg in mild cases particularly with ABO incompatibility and in all cases treated after 72 hours of age

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Iron Requirements in Infancy

II The Influence of Iron Fortified Cereals Given during the First Year of Life on the Red Blood Picture of Children at $1^1/_2$–3 Years of Age

by PETER JOHAN MOE

Introduction

The studies to be reported in this article are an extension of the longitudinal studies of iron requirements during the first year of lif published recently [3].

We have reasons to presume that a normal infant receiving adequate dietary iron supply during the first year of life will run little risk of developing iron deficiency anemia during the following years. We have been interested in studying whether this is really true and have therefore made a follow up study of the 211 participants in the previous investigation. No attempts have been made to perform a more exact determination of the daily iron intake of the participants after the age of 1 year as this appears to be very difficult in such a large series above the age of one year

An attempt has also been made to determine the incidence of iron-deficiency anemia in Norwegian children during their first years of life and the influence of different types of infant cereals commonly used in Norway on the red blood picture of the subjects at the age of 1 to 3 years (control series).

Further an attempt has been made to evaluate the long term effect of iron therapy in anemic infant

Literature

A survey of the literature has been given previously [3]. The only article of special interest which has appeared since that survey is by Beal and coworkers [1], who claim that a diet which provides 0.5 mg iron/kg or more daily to infants during the first year of life was adequat t meet the iron requirements for hemoglobin synthesis. However these studies comprised only 59 infants, which is too small a number for a longitudinal study of iron requirements. All the infants had recei ed iron fortified cereals, and it is evident that the reported figures for daily iron intake must be too low The infants had been under the care of different practicing physicians, and no iron analyses had been performed on the diets.

Clinical Material

The present study was performed on 300 healthy children aged $1\frac{1}{2}$–3 years, during the period October 29 1962 t April 4, 1963.

An attempt was made to perform follow up study on all the 11 infants participating in the longitudinal studies of iron require-

TABLE 1 *Reasons for non participation in the follow up studies*

	N of cases
Moved from district	16
Treated by another technique	3
Died from leukemia	1
Failed t keep up attendance	9

ments during the first year of life (groups A, B and C) Twenty-nine of the 11 infants (13 7%) did not participat in this part of the study for reasons shown in Table 1

Almost all other children in the age group 1½–3 years attending our well baby-clinic during the period October 29 1962 to April 4, 1963 were also studied (control series) Most of these 118 children attended our well baby-clinic regularly All of them had re ceived ordinary infant diets under the super vision of a w ll-baby-clinic Twenty of the 118 children belonged to group D in the previous study [3]

The Diets

All the 300 children included in this report had received a very similar dietary regimen uring their first year of life using the re-commendations for infant feeding commonly used in Norway Thus, the variable factor in the liet was the iron content of the cereals. Details for the diets of the infant in groups A, B nd C as well as the result of iron analyses of the constituent of the diets, have been given in the monograph [3] Detailed dietary information was not obtained for the children in the control series, but the tot l daily ron intak during the first year of lif can be estimated for most of the hil dren (see later)

The children in the control series were di vided into the following 5 subgroups, based on the types of cereal used luring the first year of life

Group I consist of 7 children who received whole wheat cereal ("hvetegrupp) regularly during the f rst year of lif None f them had received any type of iron-fortified ce reals.

Group II consists of 19 children who had received non-fortified cereals, and for some periods cereals with a low iron content ("5 mg" cereal) as well.

Group III consists of 31 children who had received iron fortified cereals w th low iron content ("5 mg" cereal), and no other types of cereals.

Group IV consists of 44 children who had received iron-fortified cereals with a high iron content (15–40 mg per 100 g cereal) for a shorter or longer period of time plus other types of cereals.

Group V consists of 17 children who had received only iron-fortified cereal with a high iron content.

The children in group III received the same type of iron fortified cereals as the children in group C from the longitudinal studies, but on an average they had probabl received less cereal than the latter Their total daily iron int ke must therefore have been maximum 5.4–8.4 mg The children in groups I and II must have recei ved a signi ficantly lower dietary iron intake during their first year of lif than the children in group C (and group III). Infants on ordinary unsupplemented diet (group I) will reach a total daily iron int ke of 3–6 mg during the last month of the first year of lif [3].

It is not possible to gi e exact figures for total daily iron int ke of the h ildren in group IV as there must ha isted large ranges depending on thei intake of iron fortified cereals with a high iron content. The total daily iron int k of the children in group V is estimated to ha e been 10–?0 mg during the last month of the f rst yea of lif

No iron fortified cereal were supplied after the age f 1 year and all the 3?) hil dren recei ed the same supers sion al dietary instructions from then onwards. How tine dietary histories were obtained on all the children t each well baby-clinic sit and more complet dietary history was btained for each hild d ring the period for this study Attention was paid t letect an gross deviation from usual feeding habits,

particularly any increased milk consumption. It was also important to get exact information regarding supplementing the diet with iron.

Methods

The infants stat of iron nutrition was evaluated by studying the hemoglobin concentration and erythrocyt morphology as in the first part of this investigation. The only change in methods was the use of a Ljungberg Celloscope in counting red blood cells, instead of the Zeiss counting chamber ad modum Bürker The two methods have previously been compared [3], and there was a close correlation between the figures obtained. All the hematocrit readings and RBC count were performed by the technician, Miss Helene Lassen. She also checked the hemoglobin readings on the last ½ of the samples.

Age f the Children

Almost all the children from the longitudinal studies were between 2 and 3 years of age at the time of this investigation. Sixty-one of the 118 children in the control series were between 1½–2 years of age and the rest were 2–3 years of age The normal red blood picture changes very little from the age of 1½–3 years, and the lower limit of normal hemoglobin concentration will, therefore probably be the same throughout the whole period.

The mean ages in all the groups were bove years. Most of the children in the control series are either a littl younger or older than those in the longitudinal studies, since the longitudinal studies comprised all healthy children attending the well-baby-clinic t the age of 3 months during a given period of time.

Results

Influence of iron fortified cereals given d g the first year of life on the red blood picture of children at 1½–3 years of age

The mean blood values of the 182 children from the longitudinal studies and the 118 in the control series aged 1½–3 years, are presented in Table *

Groups A and B in the longitudinal studies and group V in the control series comprise children who have received an adequate daily iron intake during the first year of life "Student s *t*-test" shows that those children, 131 in all, have a highly significant higher hemoglobin concentration ($P < 001$) than the children in groups C, I II and III—123 in all—who had received an inadequate daily iron intake during the first year of life in spite of the fact that many of the latter children had received courses of iron therapy Group IV

TABLE _ *Mean blood values children aged 1½–3 years*

	Group	N of cases	Hb. g %	Hem.	MCHC
Longitudinal studies	A	51	11 78	38.9	30.1
	B	63	11 8	38.8	30.4
	C	63	11.82	38.8	29.9
Control series	I	7	11 13	37.8	29 6
	II	19	11.23	38.1	29.7
	III	31	11.22	38.2	29.6
	IV	[illegible]	[illegible]	[illegible]	[illegible]

TABLE 3 *Information about previous iron therapy and distribution of hemoglobin concentrations at 1½–3 years of age*

Group	Total no. of cases	No. of cases receiving iron therapy	No. of cases with Hb. (g %)		Iron therapy or Hb. below 11	
			< 10.5	10.5–10.95	No.	% of cases
I	7	3	1	1	5	5/7
II	19	4		3	8	42
III	31	5	3	7	13	4
C	68	19	7	6	26	38
IV	44	4	1	4	7	16
B	63		1	0	2	3
V	17	1	0	0	1	6
A	51	1	1		4	8

occupies an intermediate position, both with respect to dietary iron intake during the first year of life and blood values.

Table 3 demonstrating the number of children in each group who had previously received courses of iron therapy and the distribution of hemoglobin concentrations at 1½–3 years of age is more interesting. The groups have been arranged according to dietary iron intake during the first year of life.

Table 3 demonstrates that 5? of the 123 children in the first four groups had either received iron therapy or had hemoglobin concentrations below 11 g per 100 ml at 1½–3 years of age whereas the corresponding figures for the last three groups were only 7 out of 131 children.

Table 3 also shows the marked difference between the children in groups A and B and those in group C (longitudinal studies) as well as a corresponding difference in the control series between the children (group V) receiving an adequate iron intake during the first year of life and the children (groups I, II and III) receiving significantly less than 10 mg iron daily during the latter part of the first year of life.

The 7 children in group I received a "good" non fortified diet during the first year of life. It is a small group but 5 of the 7 children had either received iron therapy or had hemoglobin concentrations below 11 g per 100 ml at 1½–3 years of age. The 19 children in group II received very nearly the same dietary iron intake as the children in group I. Eight of the 19 children had either received iron therapy or showed hemoglobin concentrations below 11 g per 100 ml at 1½–3 years of age. There can be little doubt therefore that as stated in the literature [?, 3] even a good non fortified diet during the first year of life may provide less than the required amount of iron.

Information about blood valu s of the children prior to iron medication

An objection to Table 3 may be that it contains no information about the indication for previous iron therapy. This information was given in the case of children participating in the longitudinal tudies (Moe [3], page 35 1963) as was the response to iron therapy. The indications for

TABLE 4 *Blood values of children in the control series prior to iron therapy and at 1½–3 years of age.*

Infant No.	Group	Before iron therapy Hb. g %	Hem. %	RBC mill/mm	At 1½–3 years of age Hb. g %	Hem. %	RBC mill/mm
222	I	7.25	31	4.74	10.6	36.5	4.47
229	II	8.8	33	4.81	11.2	36	4.41
238	III	9.75	34.5	4.67	10.9	37	5.02
232	II	10.05	34	3.44	11.35	39	4.80
233	II	10.5	36	4.09	11.8	36	4.46
316	IV	10.4	34	4.20	10.95	41.5	4.00
241	III	10.55	36	4.25	11.55	39	4.40
319	IV	11.65	40.5	3.98	11.3	37.5	4.70
258	II	8.3			10.96		4.60
267	III	9.4			12.1	40	5.10
281	III	10.0			10.55	34.8	3.76
248	I	10.8	(Routine laboratory)*		11.35	37	4.30
246	I	10.7			11.35	38	4.38
293	IV	11.0			11.85	38	4.71
310	IV	11.1			9.8	35	4.60
333	V	11.6			12.65	41	4.85
270	III	?			10.95	41.5	4.00

* These tests were performed by different technicians on our routine laboratory the same Ljungberg colorimeter being used as in the main studies.

giving iron therapy to the children in the control series appear from Table 4. It may be seen from T ble 4 that 4 of the 5 children belonging to groups IV and V had relatively high hemoglobin concentrations when iron therapy was started, namely 11 g per 100 ml or more. The indication for giving iron medication to these 4 children is questionable

The long term val e of a short course of iron therapy n anemic infants

Table 4 also shows that 7 of the 17 children in the control series receiving iron therapy before the age of 1½ years had hemoglobin concentrations below 11 g per 100 ml during their 1½–3 years age period. Seven of the l children from the longitu dinal studies [3] who received iron therapy before the age of 1½ years had hemoglobin concentrations below 11 g per 100 ml when aged 1½–3 years.

Most of the children received iron therapy for about 4 weeks. This is too short a period of treatment to fill the iron stores. The frequent recurrence of low hemoglobin concentrations demonstrates the inadequacy of a short course of iron therapy in preventing iron-deficiency anemia com pared to the use of iron-fortified cereals with an adequate iron content during the first year of life

Children with low hemoglobin concentrations at 1½–3 years of age and the effect of iron therapy

There were no cases of more severe iron-deficiency anemia at the age of 1½–3 years. This is probably due to th fact that many of the children receiving a low daily iron

TABLE 5 *Blood values of 1[illegible] children with hemoglobin concentrations below 10[illegible] g per 100 ml aged 1½–3 years and the effect of iron therapy*

Infant no.	Sex	Group	Before iron therapy Hb. g %	Hem. %	MCHC %	After iron therapy Hb. g %	Hem.	MCHC
59	M	A	9.65	37	26.1	11.85	42	[illegible]
186	F	C	9.85	35	28.1	11.5	39	[illegible]
219	M	IV	9.9	33.5	29.5	11.05	37	31.6
123	M	B	9.9	36.8	[illegible]	11.1	39	[illegible]
207	M	IV	10.0	36.6	27.4	[illegible]	38.5	30.[illegible]
176	M	C	10.[illegible]	35	[illegible]	11.6	34.5	31.9
11	M	C	10.[illegible]	36	[illegible]	11.15	31	[illegible]
265	M	III	10.25	37	27.8	11.4	39	[illegible]
150	M	C	10.4	36	[illegible]	11.2	41	[illegible]
4	M	I	10.45	35	29.9	11.2	35	[illegible]
183	M	C	10.45	36	[illegible]	11	35	33.[illegible]
281	M	III	10.55	34.5	30.4	11.05	33	33.5
264	M	III	10.6	36	29.4	11.15	36.5	30.[illegible]
222	M	I	10.6	35.5	29.9	[illegible]	[illegible]	32.0
164	F	C	10.6	38	27.9	10.9	39	[illegible]
40	F	II	10.65	37	28.8	12.1		
179	M	C	10.65	37	[illegible]	[illegible]	35.5	[illegible]

intake during the first year of life had received iron therapy at the age of about 1 year. Blood values of 17 children with hemoglobin concentrations below 10.7 g per 100 ml aged 1½–3 years and the effect of iron therapy is shown in Table 5. Nine children with hemoglobin concentrations below 10.7 g per 100 ml could not for various reasons be checked after iron therapy had been instituted. Their mean hemoglobin concentration was 10.2 g per 100 ml, and all but one (from group IV) belonged to groups I, III or C.

All but one of the [illegible] children with hemoglobin concentrations below 10[illegible] g per 100 ml had hematocrit of 37 or below and all but 5 had MCHC below 30. Table 5 shows that 15 of th[illegible] 1[illegible] children achieved hemoglobin concentration of 11 g per 100 ml or more after 4–8 weeks of iron therapy. The increase in hematocrit wa[illegible] not a[illegible] consistently good.

Six of the 16 children with hemoglobin concentrations of 10.7 to 10.95 g per 100 ml at 1½–3 years of age had blood tests performed following iron therapy. All of them had reached hemoglobin concentration above 11.2 g per 100 ml and their hematocrits were 39 or above.

The incidence of iron-deficiency anemia in the longitudinal studies during the age period 1–3 years

Table 6 lists the number [illegible] children in the *longitudinal studies* [illegible] h[illegible]ing h[illegible]moglobin concentration below 11 g per 100 ml at the age of 1 or 1½–3 years in order t[illegible] give an impression of th[illegible] numbers of children from th[illegible] diff[illegible]rent groups wh[illegible] developed signs of iron deficiency anemia during their first years of li[illegible]

Table 6 show[illegible] that 14 of th[illegible] [illegible] children (20.0[illegible]) in group C had hemoglobin concentration below 10.[illegible] g per 100 ml [illegible]

TABLE 6 *No of cases in the longitudinal studies with hemoglobin concentrations below 11 g per 100 ml at the ages of 1 or 1½–3 years*

Group	Total no. of cases	No of cases with Hb. (g %) Below 10	10–10.45	10.5–10.95
A	51	1	0	3
B	63	1	0	2
C	65	4	10	15

only one in each of the other two groups had such low hemoglobin concentrations Those two children will be discussed briefly Case 59 (group A) had a hemoglobin concentration of 11 8 g per 100 ml at the age of 1 year This child received large quantities of milk during his second year of life, about 1 liter per day and he developed the typical features of iron-deficiency anemia with a hemoglobin concentration of 9 65 g per 100 ml at th age of .. years. The second child, Case 123 (group B) had a hemoglobin concentration of 10.8 g per 100 ml at the age of 1 year and he was the only one who did not respond to iron therapy at that age The reason for this child s persistently low hemoglobin concentration from the age of 9 to 23 months, in spite of high daily iron intake during the first year of life and prolonged iron therapy at the age of 1 year is uncertain.

A closer study of the 15 children in group C with hemoglobin concentrations of 10.5–10.95 during the age period 1 3 years, discloses that many of them must have been anemic, and the total incidence of iron-deficiency anemia in group C during the age period 1 3 years was probably nearly 35 %.

TABLE 7 *Number of ch ldren in the control series with hemoglob n concentrations below 11 g per 100 ml during the age period 1–3 years*

Group	Total no. of cases	No. of cases with hemoglobin (g %) Below 10	10–10.45	10.5–10.95
I	7	1	1	3
II	19	3	2	4
III	31	3	2	6
IV	44	1	2	3
V	17	0	0	0
Groups I V	118	8	7	16

The ncidence of iron-deficiency anemia among children in the control series during the age period 1–3 years

Table 7 lists the number of children in the control series showing hemoglobin concentrations below 11 g per 100 ml during the age period 1–3 years.

Table 7 shows that 15 of the 118 healthy" children (12.7 %) from our well baby-clinic were definitely anemic during the age period 1–3 years with hemoglobin concentrations below 10.5 g per 100 ml. The number would have been even higher if all the children had been followed to the age of 3 years, and especially if all the children had remained untreated. A closer study of the 18 children with hemoglobin concentrations of 10.5–10.95 shows that many of them must have been anemic and the total incidence of iron-deficiency anemia during the age period was probably approximately 25 %. All the anemic children were in groups I–IV and 27 of the 57 children (47 %) in the first three groups had hemoglobin concentrations below 11 g per 100 ml during the age period 1–3 years.

Role of Infection

Only one child had an *infection* which definitely influenced the blood values. Case 85 (group B) ran a high *fever* with glandular swelling in the neck and infectious blood picture at the age of 26 *months*. Her hemoglobin concentration was 10.6 g per 100 ml, but increased without iron therapy to 11.05 g per 100 ml. One further child deserves closer comment i.e. Case 2?0 (group III). This child had always had a tendency to diarrhoea and one cannot exclude the possibility that this has influenced his blood picture.

This part of the investigation confirmed the impression from the longitudinal studies that infections are of *minor* importance as a cause of anemia in this age period compared to the role *of the dietary iron intake*.

Discussion

An infant approximately trebles his *weight during the first year of life* whilst weight increment during his second year of life is about 2.5 kg or about 25 % of the weight at the age of 1 year. The high concentration of hemoglobin and the relatively *large* blood volume at birth are the major contributing factors in reducing the exogenous iron requirements during the first year of life, but a normal infant poorly supplied with endogenous iron will need to increase his iron content by 2-300 % during the first year of life [4]. The hemoglobin concentration will normally remain practically unchanged *during the second year of life* and it would seem justified to presume that an infant (with a normal hemoglobin concentration at the age of 1 year) will need to increase his total body iron by about 25 % during *the second year of life*. *Exogenous* iron requirements during the second year of life will, therefore presumably be smaller than during the third and fourth trimester. *In spite* of this most recommendations as regards daily iron allowances stipulate larger intakes in the second year of life. *It is difficult* to perform an exact determination of daily iron intake in a large number of infants after the age of 1 year. Theoretical calculations of iron requirements can only serve as rough guides, but it is likely that a good nonsupplemented diet will meet the iron requirements after *the age of 1 year* and one may therefore presume that an infant with a good state of iron nutrition at the age of 1 year will run little risk of developing iron-deficiency anemia during the following years, on an ordinary diet (with a daily iron intake of about 6-7 mg).

The studies reported in this article show that the regular use of iron fortified cereals with an adequate iron content (groups A, B and V) during *the first* year of life will almost completely prevent the occurrence of iron-deficiency anemia during the first years of life.

No reliable *figures* exist as *to the* approximate incidence of iron-deficiency anemia during the first years of life in Norway. These *studies indicate* a relatively high incidence of mild or moderate iron deficiency anemia among healthy *infants* attending regularly a well-baby-clinic in Oslo. There can therefore be no doubt about the desirability of a change in dietary iron intake during the first year of life in order to secure an adequate iron intake *for* all healthy infant and thereby prevent the development of iron-defi-

ciency anemia in infancy and early child hood.

The inadequacy of non-fortified diets during the first year of life in preventing iron-deficiency anemia has been demon strated. Iron-fortified cereals are now in common use all over the country although there probably is a higher percentage of infants receiving iron-fortified cereals in Oslo than in rural districts All iron-forti fied cereals used in Norway today have an adequate iron content, provided the infants are fed according to the feeding instructions. It has been maintained that it is unnecessary to give iron-fortified cereals twice a day provided the cereals have a significantly high iron content, but this question has not been solved in the present investigation. Most infants are fed cereals twice a day until the age of 9–12 months, and it is not quite obvious why they should not be given iron-fortified cereals twice a day Iron-fortified cereals do not represent a large burden on the family budget. They are much more im portant for the prevention of iron-defi- ciency anemia during the first year of life than are canned meats, vegetables and fruits. All the 17 children in the control series (group V) receiving only iron forti- fied cereals with a high iron content (15–40 mg per 100 g of cereal) had high hemoglobin concentrations at 1½–3 years of age but there were several anemic chil dren among the 44 children (group IV) receiving iron fortified cereals with a high iron content, alternating with cereals with a low iron content The children in the control series did not participate in the longitudinal studies, and they were not subjected to constant dietary supervision. It is, therefore impossible to know the precise composition of their diets during their first year of life but the above ob- servations demonstrate the risk involved in permitting the use of iron-fortified cereals alternately with cereals of a low iron content

Summary

The present study was performed on 300 healthy children aged 1½–3 years. One hundred and eighty two had participated in previous longitudinal studies of iron requirements in infancy the other 118 belonged to a control series of healthy children attending a well-baby-clinic The latter children were divided into 5 sub- groups based on the types of cereals they had taken during the first year of life

The incidence of iron-deficiency anemia in Norwegian children during the first years of life is still high, depending mainly on the type of cereals they have been given. These studies have demonstrated that children receiving an adequate daily iron intake during the first year of life (10 mg or more from the age of about 8 months) will run little risk of developing iron-deficiency anemia during the follow ing years on an ordinary diet

It is difficult to carry out an exact deter mination of the daily iron requirements during the second year of life From a practical point of view it is important to know that iron fortification of a good diet after the age of 1 year is unnecessary provided the infants have a good state of iron nutrition at the age of 1 year This will probably mean that the daily iron requirements during the second year of life is about 5–7 mg daily and definitely not as high as 10–15 mg daily

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Late Stages of Pulmonary Hyaline Membranes of the Newborn

by BENGT ROBERTSON RAGNAR TUNELL and ULF RUDHE

In neonates suffering from pulmonary hyaline membranes and surviving the first few days it has been observed that the membranes become infiltrated by histiocytes, fragmented and detached from the alveolar walls. This has been interpreted as evidence that the membranes are being removed [3 10 15 18] In these cases a cellular reaction has also been described in the alveolar walls proper proliferation of the alveolar epithelium and accumulation of fibroblasts. These latter features have been interpreted as a process of reparation, following previous damage to the alveolar walls and it has been postulated, that such a damage probably is related to the development of hyaline membranes [3 15] Studies with the lec tron microscope have revealed destruction of the alveolar lining and swelling of the endothelium in capillaries beneath the membranes in an early stage of the disease [5 0] No permanent sequelae however have been recognized in the surviving cases [11] The report hitherto published on the structure of pulmonary hyaline membranes in the neonatal period are confined to the first postnatal week.

Th purpose of this study is to present the morphologic features in the lungs of four neonatal cases of idiopathic respira tory distress surviving the first postnatal week. Three of these patients died at the age of 13 1 and 23 days, respectively The fourth infant survived and is still living at 16 months of age In this case a lung biopsy was performed at the age of 8 week

Case Reports

Case I

Clin cal h story This concerns a prema turely born male infant (birth weight 1700 g), the first child of a healthy 22 year-old mother After an uneventful pregnancy of 32 weeks the infant was born in normal spon taneous vertex delivery The immediat neonatal course wa uncomplicated. Cyanosis, however still persisted after 10 minutes. At this time slight respiratory distress was observed, which gradually increased. At the age of three hours the Silverman score w 5 and the respiratory frequency 80/min. Generalized cyanosis was present but dis appeared after the administration of 50 oxygen in the inspired air

During the subsequent four days the respiration was highly irregular There were frequent episodes of pnea but the respira

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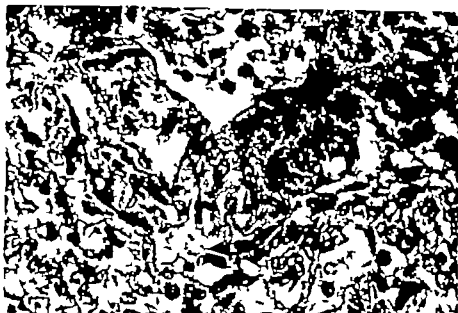


Fig 1 Alveolar duct filled with cellular acidophilic material. Premature infant (birth weight 1 00 g), aged 13 days. Idiopathic respiratory distress syndrome in the neonatal period. (Case 1.) Hematoxylin-eosin 530.

tory distress gradually decreased. On the fourth day after birth the spontaneous respiration topped for a period of 14 minutes, which made it necessary to apply the Engström respirator The setting of the respirator was adjusted according to results obt ined from arterial blood gas analyses. A comparat ly low positive pressure (35 cm H_2O) wa need 1 to maint in the required minut lume of 500 ml The general condition of the infant was good, but some respiratory distress i.e. intercostal retraction and prolonged expiration during periods of spontaneous respiration, persisted

At the age f 10 days the clinical course was complicated by diarrhea, melena, vomiting and increasing dyspnea The infant rapidly deteriorated and expired t the ge of 13 days.

Roentgenologic f nd gs. Roentgen examination of the chest was carried out repeatedly during the disease period A few hours after birth disseminated atelect ses of a reticulo-granular appearance were noted in both lungs. The somewhat irregular distribution had changed to a more uniform one on the next day On the fourth day the involved area had spread to include much of the periphery of the lungs. Additional progression was seen on the leventh day when multiple areas of patchy consolidation developed bilaterally The ppearance of the lungs particularly in the studies following the initial examination, was considered compatible with the hyaline membrane syndrome

The cl ical diagnose were immaturity + idiopathic respiratory distress syndrome in the neonatal period + acute gastroenteritis + pneumonia.

Gross f nd ngs at autopsy Both lungs were consolidated and weighed 24 and 28 g respecti ely (normal combined weight 30 ± 9 g). There were large septal and subpleural bleedings. The mucosa of the esophagus, the stomach and the intestines showed foci f recent hemorrhage The leptomeninges presented recent wide-spread hemorrhage in the vicinity of the cerebellar hemispheres. N hemorrhage int the ventricular system of the brain was noted The cardiovascular



Fig. 2. Thickened, partly hyalinized al eolar wall with accumulation of fibroblasts. Amorphous material in the lumen. (Case 1) Hematoxylin-eosin 530.

system was normal. The ductus arteriosus was patent. The other organs were unremarkable

Histologic findings n lung specimens (The sections were examined with the following stains: Hematoxylin-eosin, v Gieson, H i denhain Azan and Lendrum reticulum st in.) In several alveoli and lveolar ducts of both lungs acidophilic hyaline material was observed, either filling the lumina of the air-spaces or forming membranes covering the walls. This material was infiltrated by histiocytes (Fig 1). Many alveolar walls were thickened and hyalinized, with hyper chromatic nuclei in the cells of the al eolar epithelium (Fig 2). Beneath the epithelium of these al eolar walls many fibroblast and excess of reticulin fibers were observed (Fig 3) However no excess of collagen f bers could be demonstrated. There was no in flammatory exudat in the air-spaces.

Case *

Cl ical history This concerns a prematurely born infant (birth weight 1900 g), the first child of healthy 18-year-old woman. After an uneventful pregnancy of 32 weeks there was spontaneous uncomplicated deli very The child was born in vertex presentation. Immediately fter birth the infant cried vigorously Cyanosis persisted and 10 minutes after birth intercostal retractions were bserved. Three hours fter birth severe respiratory distress had developed (Sil er man score 9). There was marked generalized cyanosis, which disappeared after the administration of 80% oxygen in the inspired ir From the 12th hour after birth arterial blood gas analyses were repeatedly performed via permanent catheter in one umbilical artery During the first three postnatal days the arterial oxygen saturation aried between 40 and 60% depending upon the content of xygen in the inspired air The values of P_{aco_2} varied between 45 and 50 mm Hg and those f BF between 15 and 5 mEq/l. The respiratory frequency was 70–80/min. Because of severe sternal retractions the in fant was treated with thora elevation dur ing the first three days.

From the fourth day the respiratory distress gradually diminished. There was however a strong tendency to vomit requiring intravenous nutrition during the following weeks. On the 18th day an acel

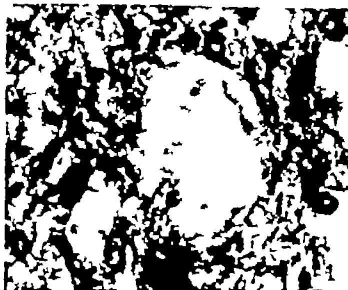


Fig. 3. Thickened alveolar wall containing excess of reticulin fibers. (Case 1.) Laidlaw reticulum stain × 530

Fig. 4. Case 2, day of birth. Small reticulo-granular densities are distributed in the hilar and basal areas bilaterally

dent with the feeding tube occurred the tube formed a loop in the esophagus and its tip came t the level of the larynx, resulting in marked aspiration and subsequent telec tasis of the right upper lobe The hild rapidly deteriorated fter this event Apneic spells occurred and in spit of the application of the Engström respirator the child died two days later t the age of 1 days During the last day there were bleedings from the gastro-intestinal tract

Roentgenologic find ngs. Roentgen examination of the chest carried out on the day of birth demonstrated wide-spread, regularly distributed dense areas f reticulogranular ppearance in both lungs (Fig 4). The main bronchi and some of the larger subdi isions were clearly seen against the contrasting dense parenchyma. The pattern was characteristic of the hyaline membrane syndrome. A moderat regression f the atelectases was noted on the fourth day Confluent patchy infiltrates appeared fter 18 days and were considered t be secondary to aspiration.

The cl ical diagnoses were immaturity – idiopathic respiratory distress syndrome in the neonatal period + pneumonia (aspira tion).

Gross f nd ngs at autopsy Both lungs were consolidated with foci of bronchopneumonia. The lungs weighed 30 and 35 g, respectively (normal combined weight 44 ± 13 g). There was purulent material in the trachea and the main bronchi. The mucosa of the distal part of the esophagus was partly ulcerated. The remainder of the gastro-intestinal tract was normal. There were no cardiovascular abnormalities. The ductus arteriosus was patent. The other organs, including the brain, showed nothing remarkable

Bacteriologic f nd ngs Pseudomonas aeruginosa was cultured from the lungs.

Histologic findings i l ng specimens (The sections were examined with the following stains: Hematoxylin-eosin, v Gieson, Heidenhain Azan and Lendrum reticulum stain.) Foci of bronchopneumonia were present in both lungs. In many al eoli amorphous material was found indicating aspira-

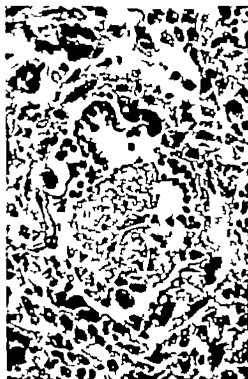


Fig 5. Thickened, partly hyalinized alveolar wall Hyperchromatic nuclei in the alveolar epithelium. Amorphous material and some erythrocytes in he lumen. Premature infant (birth weight 1900 g), aged 21 days. Idiopathic respiratory distress syndrome in the neonatal period. (Case 2) Hematoxylin-eosin 425.

tion. There were no hyaline membranes. Many alveolar walls were thickened and hyalinized in the same fashion as in Case 1 the nuclei of the alveolar epithelium were hyperchromatic and many fibroblasts were observed in the interstitium. In this case in contrast to Case 1 there wa excess of col lagen as well as reticulin fibers in the thicke ned alveolar walls (Figs. 5–7).

C se 3

Cl ical history This concerns prematurely born male infant (birth weight 1320 g), the first child of a 29-year-old healthy woman. On account of acute abdominal symptoms ppendectomy was performed in



Fig 6. Thickened alveola wall, containing fibroblast and excess f collagen f bers (arrow). (Case _) Gieson 470.

the sixth month of pregnancy The appendix, however was normal, and the clinical symtoms were attributed to pancreatitis. After a pregnancy of _8 weeks the child was born in spontaneous normal vertex delivery Within a few minutes after birth there was moderate respiratory distress (Silverman score 5) and light cyanosis. After administration of 35 _ oxygen in the inspired air the cyanosis disappeared. During the first few days of lif the dyspnea gradually improved but the respiration was irregular and mainly of the Cheyne-Stokes type Apneic spell occurred repeatedly and seemed to be provoked by oral feeding Therefore the infant was given intravenous nutrition. During longer period of pnea artificial ventilation with intermittent positive pressure had to be performed, on one occasion for a period of 30 minutes.

The general condition of the infant gradu lly deteriorated During the last days of lif cerebral symptoms were prominent, con vulsions occurred there were muscular hypotonia and few spontaneous movements. The respiration rat decreased to *0–30/min. Comparati ely long periods of apnea (<10 min.) supervened frequently The body weight had decreased to 1010 g when the inf nt died t the age of 24 day



Fig Detail from the same alveolar wall as represented in Fig 6. The collagen fibers are clearly visible (Case 2) v Gieson 1320

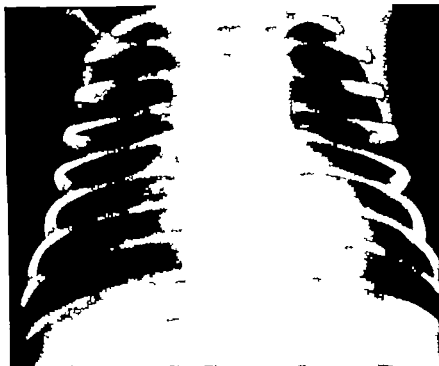


Fig. 8. Case 3, three hours after birth. Dense areas of reticulo-granular appearance are scattered centrally and basally. The air-filled bronchi are well defined.

Roentgenologic findings. Roentgen examination of the chest performed three hours after birth showed areas of atelectasis of reticulo-granular appearance in both lungs. The central and basal portions of the lungs were predominantly involved. The air-filled bronchi were outlined against the surrounding dense parenchyma (Fig. 8). The appearance of the lungs was essentially unchanged during the following five days. Progression was later observed. The abnormal pattern of the lungs and the sequence of findings were considered to be consistent with the hyaline membrane syndrome.

The clinical diagnoses were immaturity + idiopathic respiratory distress syndrome in the neonatal period, convulsions, probably secondary to hypoxic cerebral damage.

Gross findings at autopsy. In both lungs the parenchyma was partly emphysematous, partly consolidated and there were foci of bronchopneumonia. The combined lung weight was 38 g (normal 30 ± 9 g). There were many petecchiae in the pleurae and the pericardium. The cardiovascular system was otherwise normal. The ductus arteriosus was closed. Wide-spread hemorrhage in the leptomeninges was observed and there was a recent thrombus of the superior sagittal sinus. No macroscopic evidence of damage to the brain parenchyma could be recognized.

Bacteriologic findings. Pseudomonas aeruginosa was cultured from the lungs.

Histologic findings — lung specimens (The sections were examined with the following stains: Hematoxylin-eosin, v. Gieson, Heidenhain Azan and Lendrum reticulum stain.) In both lungs there were foci of bronchopneumonia and intra-alveolar hemorrhage. Many thickened alveolar walls of the same type as in Case 1 and 2 were observed. In these alveolar walls fibroblasts and excess



Fig 9 a. Case 4 One hour after birth. Small atelectases are diffusely distributed throughout both lungs.

of collagen as well as reticulin fibers could be recognized There was no evidence of hyaline membranes.

Case 4

Clinical history This concerns a prematurely born male infant (birth weight 1900 g) second child of a healthy 23-year-old woman. Following an estimated gestational period of 28 weeks the child was delivered by caesarean section, because of profuse bleeding from a placenta previa At birth the infant was pale asphyctic without spontaneous breathing Resuscitation by intubation and artificial ventilation was immediately performed Four minutes after birth spontaneous breathing occurred. Intercostal retractions were present from the first breath and there was generalized cyanosis. At three hours there was severe respiratory distress (Silverman score 9) and despite the administration of 80% oxygen in the inspired air cyanosis persisted. At that time a permanent catheter was inserted into one umbilical artery Blood-gas analysis revealed an oxygen saturation of 30%, P_{aCO_2} of 75 mm Hg and metabolic acidosis (−?3 mEq/l BE) The respiratory frequency was 40/min. Five hours after birth all spontaneous breathing disappeared and there was a cardiac arrest during a few seconds. After successful resuscitation by external heart massage intubation and artificial respiration with positive pressure the Engström respirator was applied The setting of the respirator was adjusted according to results obtained from blood-gas analyses. At the age of 1? hours adequate ventilation could be maintained by administrating 100% oxygen under a positive pressure of 50 cm H_2O and a respiratory frequency of ?0/min. The oxygen saturation was 90%, P_{aCO_2} 35 mm Hg and BE −5 mEq/l in blood taken from the catheter in the umbilical artery

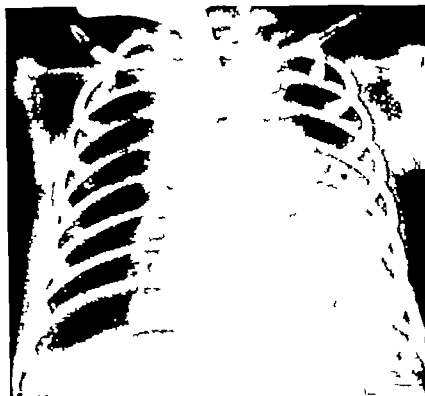


Fig. 95 Case 4. One day of age, following intubation and artificial respiration. The abnormal pattern is unchanged.

T days after birth tracheostomy was performed.

The general condition slowly improved and from the third day the oxygen content in the inspired air and the degree of positive pressure could be gradually diminished. During the first four days of lif the child was given intravenous nutrition, but there after oral feeding was started.

There was no blood incompatibility between mother and child. On the fourth day fter birth, however serum bilirubin was 25 mg%. Since the respiratory and circulatory functions of the child were still impaired no exchange transfusion was performed Audiometric studies were carried out from the 5th day of lif (Dr M. Barr) showing reduced hearing. N other linical evidence of kernicterus was recognized. The serum bilirubin level gradually decreased following the fourth day

At the age of one month the artificial ventilation could be discontinued. Moderat intercostal retractions and prolonged expirium persisted, however during the subsequent month. Decannulation was not performed until months after birth.

Because of the clinical and roentgenological findings (see below) thoracotom w per formed at the age of 56 days and small biopsy specimen was taken from the anterior segment of the right middle lobe for histologic examination. The postoperativ course was uneventful. When the child wa sent home one month later there was still slight impairment of respiration evidenced by pro-



Fig 1 Thickened al eola wall with excess of f broblast and collagen f bers. Cellular amor phous material in the lumen Biopsy specimen from premature infant (birth eight 1900 gt, ed 8 eeks. Idiopathic respiratory distress syndrome in the neonatal period. (C se 4) Gieson 450

longed expirium and rhonchi over both lung

Roentgenologic findings Roentgen examination f the chest one hour after birth le monstrated findings that were compat ble w th those f the earl stages f the hyaline membrane syndrome (Fig 9a) In the examinations performe l t j and 6 hours after birth the changes were more marked. The air-filled bronch were outlined gainst the surrounding dense parench ma. On the fol lowing day after the patient had been intubated the abnormal pattern was still prominent in all part of the lung (Fig 9b) A low but steady learing followed. Emphysematous blebs and patchy infiltrates then developed in the right lung. Following a short period f enlargement they gradually liminished in size After two months only small areas of atelectasis remained. There were no abnormal changes seen in the left lung at this time

The clinical diagnoses were Immaturity + idiopathic respiratory distress syndrome in the neonatal period + hyperbilirubinemia + questionable kernicterus

Histologic findings (Biopsy specimen at 8 week of age from the right middle lobe The sections were examined with the follow ing stains Hematoxylin-eosin, v Gieson, Heidenhain Azan and Lendrum s reticulum stain.) P rts of the lung tissue examined showed recent intraalveolar hemorrhage which was attributed to the trauma during operation. In some areas the alveolar walls presented the same type of hyaline f brous thickening as in Cases 1 3 In these alveolar



Fig 11 Another part of the same biopsy specimen represented in Fig. 10 There is prominent thickening and fibrosis of the alveolar walls. (Case 4) Gieson 450



Fig 1.— Thickened alveolar walls with excess of reticulin fibers. (Case 4.) Lendrum reticulum stain × 210

walls there was excess of collagen as well as reticulin fibers (Fig 10–12) In some alveoli amorphous material containing a few histiocytes was found, but there were no hy line membranes.

Comment

All these four premature infants deve loped shortly after birth the characteristic clinical picture of idiopathic respiratory distress syndrome i.e grunting nostril breathing severe intercostal retractions, high respiratory frequency and genera lized cyanosis [1 17] Roentgenograms of the chest showed areas of disseminated densities arranged in a reticulo-granular pattern all over the lungs It is a well established fact that these clinical and roentgenologic findings re associated with pulmonary hyaline membranes in approxi mately 80 % of the fatal cases xpiring within the first postnatal week [9 10, 13 14 16]

After the first few days of respiratory distress the four infants all improved gradually The ultimat cause of death in three of the cases was considered essen tially unrelated to their initial disease It should be pointed out however that these three patients up to their last day of life as well as the fourth surviving patient at the time of lung biopsy still howed evidence of slight respiratory difficulties inspiratory retractions were present well as a prolonged expirium

The histologic features in the lung tissue of the four cases were similar Thickened alveolar walls were covered by hyperchromatic epithelium and showed increase of fibroblasts In the three older cases (1–50 days of age) excess of col lagen and reticulin fibers could be de monstrated in these walls in the youngest there was excess only of reticulin fibers These features, apart from the develop-

ment of reticulin and collagen resemble the proliferative and reparative phenomena occurring in connection with pulmonary hyaline membranes during the first postnatal week [3, 15]. Similar features have also been described in association with hyaline membranes in influenza pneumonia [10]. The excess of reticulin and collagen fibers in the alveolar walls of our four cases is quite compatible with a late stage of such a process of reparation as referred to above.

In the patient that expired on the 13th day, hyaline membranes infiltrated by histiocytes could be demonstrated in the lung. In the other three surviving longer no hyaline membranes were present. We believe that all these infants had pulmonary hyaline membranes in the immediate neonatal period as suggested by the clinical course, but that the membranes in the older cases had disappeared or had partly been incorporated in the alveolar wall structure. As mentioned before, macrophage response including fragmentation and probably removal of the membranes has been recognized at the age of 3–4 days in neonatal cases of this condition.

Although the histologic features presented by these cases are compatible with a late stage of a reparative process in the alveolar walls probably secondary to a damage causing hyaline membranes in the immediate neonatal period, consideration must be given to the possibility that the treatment has influenced the development of the pulmonary lesions. Experiments on adult rabbits and mice have shown that prolonged exposure to 70–100 % oxygen causes alveolar damage and development of hyaline membranes [6, 7], fibrosis of alveolar walls, however, has not been reported. Newborn mice studied under similar experimental conditions have reacted differently: emphysema of marked degree has been observed but no hyaline membranes [1.]. The effects of prolonged exposure of the human neonatal lung to high oxygen levels have not as yet been analyzed. Consequently no conclusions can be drawn concerning the possible effect of oxygen therapy in our four cases. It cannot be ruled out, however, that this particular therapy, though necessary for the maintenance of adequate ventilation, might have influenced and possibly enhanced the structural alterations of the alveolar walls. This applies particularly to Case 4 which received 100 % oxygen during the initial phase of the respirator therapy.

Even less is known about the effect of artificial ventilation with high positive pressure on the human neonatal lung. Two of our cases (1 and 4) received prolonged respirator therapy, one of these (Case 4) with high positive pressure. In Case 1 artificial ventilation was administered only during the last two days of life. In Case 3 the respirator was never applied. Since the histologic features are similar in all cases, the artificial ventilation *per se* can hardly have been a significant factor in the development of the lesions in the alveolar walls.

In Case 4 emphysematous blebs developed during the respirator treatment. This complication can possibly be attributed to the high pressure ventilation. Another point of interest concerning Case 4 is that clinical and roentgenologic evidence of pulmonary disease still persisted at the age of 2 months. This is not the rule in uncomplicated cases of idiopathic respiratory distress syndrome in

the neonatal period. Other pulmonary lesions may have been present in this case though not detectable in the small biopsy specimen that was examined histologically It has to be emphasized that this biopsy specimen was obtained from the periphery of the lung

The alveolar lesions here described resemble those of infantile lobar emphysema [4] as well as those of the Hamman Rich syndrome [8] There is still no evidence however of an etiologic relationship between severe respiratory distress in the neonatal period and the early phases of these two pathogenetically obscure pulmonary disorders.

Summary

Four neonatal cases of idiopathic respiratory distress syndrome suggesting pulmonary hyaline membranes are presented. Three of the infants died at the age of 13–23 days. The fourth survived and in this case a lung biopsy was performed at the age of 8 weeks. The histologic features in the lung tissue of all four cases were similar and consisted of thickened alveolar walls showing increase of fibroblasts and excess of reticulin or collagen fibers. These findings are consistent with such a process of repair as has been observed in the first postnatal week in cases of pulmonary hyaline membranes. Hyaline membranes infiltrated by histiocytes were still present in the case that expired at the age of 13 days, but not in the three cases surviving longer Prolonged respirator therapy was performed in two cases, in one of these with high positive pressure All cases received supplemental oxygen, one case 100 % oxygen during the initial phase of the disease. The possible effect of this therapy on the development of the lesions in the alveolar walls is discussed.

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Studies of Urinary Tract Infections in Infancy and Childhood

III Quantitative Estimation of Cellular Excretion in Unselected Neonates

by KNUT LINCOLN and JAN WINBERG

Introduction

The diagnosis of urinary tract infections mainly rests upon the demonstration of a pathologic bacteriuria and leucocyturia Accuracy in diagnosis thus requires a definition as clear as possible of the normal limits of urinary cellular and bacterial excretion. As a basis for the diagnosis of neonatal infections bacterial and cellular excretion have been established in about 600 unselected newborns. A report of the bacteriological part of the study has been published earlier [5] and demonstrated that accepted adult and childhood ranges for bacterial excretion were not applicable in neonates unless special cleaning procedures were applied. In this paper the urinary cellular excretion pattern will be reported. The results may to a certain extent explain the conflicting findings of earlier uthors [1 4] dealing with neonates.

M terial and Methods

The material has been presented in det l in previous paper [5]. The urine specimens xamined in thi study are fewer than in the former one This is due to circumstances working t random, and not the consequence of selection. The handling of the urine and different procedures for cleaning of the genital tract before collection of urine were also reported earlier Since the counts of the different types of cells were not ppreciably influenced by different methods of genital cleaning all urine specimens whether obtained after ordinary cleaning of the genital tract or after irrigation of praepuce or vulvar region, are presented together in the figures.

The cells in one cubic millimetre of uncentrifuged and unstained urine were counted in a Fuchs Rosenthal counting chamber using the highest dry bject ve (40). In some instances—see below—the urine was stained with the Sternheimer Malbin stain [9] or with methylene blue Gram-stained smears f urine or urinary sediment were also examined in these instances.

In the urine specimens mainly three dif ferent types f cells were found: leucocytes, squamous epithelial cells and non-squamous epithelial cells.

The shape of the non-squamous epithelial cells usually distinguished them from the leucocytes, although the size of these two types of cells was about equal (Fig 1)

The lassification of some oval cells was, however difficult Cells with a smooth

This investigation was supported by grant from The Statens Medicinska Forskningsråd

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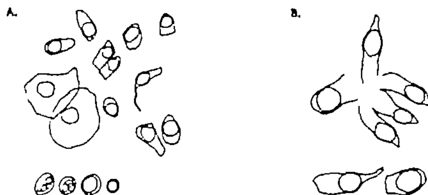


Fig 1 Line dra ings after colour microphotographs of Papanicolaou-stained alcohol-ether fixed smears of rinary sediment from 6-day-old boy *A* Two squamous epithelial cells (transitional cell) and n merou non-squamous epithelial cells. Not polymorphism, elongation, angula appearance nd similarity to ylindrical or cuboid epithelial cells. One d bious cell t the right of the squamou cells. Bottom row: four leucocytes drawn t scale *B* The cells sometimes ppear in clusters.

rounded contour w re usually classified leucocyt s while cell with a more angular hape were characterized a non-squamou ep thelial cells. Th i judgement was some times ubtl but since ich dubious cell were relat ly ncommon misinterpret tions probably ha e not appreciably in fluenced the result of the cell cou ts.

In most of these specimens th classifica tion wa facil tated b the bove mentioned t ining procedu res, which permitted a reliable separation of the polymorphon iclear leucocytes.

Result

The results of the tudy are presented in Fig. 2 3 and 4 Ea h figure shows the excretion of one type of cell and compares males and females

Leucocytes (Fig 2)

In both boys and girls most urine specimens contained between 0 and 0 leucocytes per mm³ of uncentrifuged urine It is however obvious that in the girls there are more specimens with high counts than in the boys. Thus 51 specimens out of 404 among the girls contained more than ‥ cells per mm³ of uncentrifuged urine and 19 out of 404 more than 50 (Table 1) Among the boys corresponding figures were 7/344 and */344 If those samples are excluded which contained at the same time large amounts of coliform bacteria The excretion during the first four days may be a little higher than that during the following 5–15 days.

In male urine specimens with more than 50 leucocytes there were usually at the same time a high count of coliform bac teria In Fig. 2 such specimens are represented by black dots Probably there was a urinary tract infection in these patients —as was discussed in detail in the previous paper Among the girls with a leucocyturia amounting to 50/mm³ or more there was on the other hand not a single case with high count (>100 000 bact. per ml) of coliform bacteria. A certain association to high counts of not identified slow growing bacteria of vaginal flora type was however found in these cases (Table ‥). Thus in girls especially those with more than 50 leucocytes per mm³ of

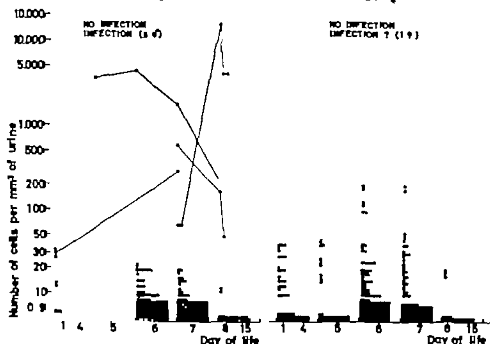


Fig. — Leucocytes in the urine of 228 newborn boys and 48 newborn girls. Black symbols notify specimens where leucocyturia was combined with marked coliuria (see 5). Half black symbol stands for specimen containing 100–200,000 bacteria of other types than coliforms. There is an obvious sex difference

TABLE 1 *Leucocytes in the urine of 4 6 neonates*

Day of life	Boys: Frequency of specimens with ≤5 cells per mm³ uncentrifuged urine	Girls: Frequency of specimens with > 25 cells per mm uncentrifuged urine	Girls: Frequency of specimens with > 50 cell per mm³ uncentrifuged urine
1–4	5/38	11/62	1/62
5	0/15	6 40	/40
6	0/136	16/158	8/158
7	–/127	18/122	8/122
8–15	0/27	0/22	0 22
Total	7/344	51/404	19 404

pressure in the right arm wa 105/75 in the left 95/75 and in the legs 1*0 80 The FCG wa repeatedly normal X ray of the heart showed a slightly increased volume (405 ml per square meter of body urface) but no definit abnormalities in configuration Congenit l heart disease was considered unlik l

Lungs The lungs were normal on X ray

Abdom l organ The spleen, which had been palpable since birth could till be f lt 4 cm below the left costal margin, and the liver about cm below the right costal margin

G n to- ary tract The penis wa normal, and the scrot l sac mall but otherwise normal Testes of prepubertal ize and con i stency could be palpated bilaterally at the xternal inguinal ring The prostate wa not palpable In M rch 1961 a right inguinal herniorrhaphy wa done The right testis ppeared normal nd a biopsy specimen was obtained

Skeleton X-ray film of the skull and extremities revealed no bnormalities.

N rvous syst m Mentally the boy was retarded he was regarded a an imbecile with ment l ge of years as assessed by a T rman Merrill test and j according to a Buhler Hetzer test Neurological examination revealed no bnormalities Ophthalmoscopic examination showed tortuous vessels in pale retina. EEG howed n bnormally *slow activ ty but no ep leptogenic burst*

Laboratory tests Routine test on blood and urine were repeatedly normal The serum potassium serum calcium serum phosphorus serum alkaline phosphatase serum cholesterol serum total lipids, and fasting blood glucose concentration were normal. The total serum protein was 7 0 g/100 ml with a normal paper lectrophoretic pattern The urinary excretion of gonadotrophin was less than 6.5 MU/24 hours (normal) Paper chromatographic examination f the urine revealed no abnormal aminoacids

Case 2 M. B., a boy was born June 2nd 1956 to a 31 year-old mother and a 38 year-old father A sister born in 1958, and both parent are healthy No other member of the f mily has had signs or ymptoms of the kind presented by the boy

The boy was born at term after an uneventful pregnancy and deli ery His birth w ight was 3050 g and his length t birth 49 cm During the neonatal period he was found to be hoarse and initially he had also difficulties in sucking From an early age the mother noticed that he was shorter than other boys of his age His psychomotor development was relatively normal he was able to sit without support t the age of 7 8 months, he w lked without support at 15 month of age he started to t lk single words t 1 months, and could speak hort sentences at years of age However at the age of 5 his diction was still indistinct and he could not roll his s. Hi mother has regarded him as being more easily exhausted by play than other boys of his age

At the ge of 3 years the boy was referred to the Department of Paediatrics at the County Hospital, Karlstad H was short and peculiar looking, with enlarged head hort neck broad flat nose bridge short broad hand and feet and ery thin hair A systolic murmur was heard. An air-encephalogram revealed slight central atrophy of both the lateral ventricles, and the basal cisterns were wider than normal. His mental development was regarded as normal

At the age of 5 years the boy was admitted t the Department of Child Psychiatry in Karlstad where a performance test showed IQ 103 In 1961 he w referred to the Department of Paediatrics, University Hospital, Uppsala, for further examination

Examination at the age of 5½ years disclosed several anomalies (Fig *). The boy was only 99 cm tall (about 4 cm less than − SD), and weighed 16.5 kg (normal for the height) The skull circumference was 51 cm (normal) The hair showed several large whorls. The nose bridge wa broad and flat. The neck was short, with moderately pronounced bilateral webbing The external ears were low-set and showed light anomalies The palate was highly arched There were no dental malformations. Hypertelorism slight e ophthalmos, and bilateral

dent with the feeding t be occurred, the tube formed loop in the esophagus and its tip came to the level of the larynx resulting in marked aspiration and subsequent at lec tasis of the right upper lobe The child rapidly deteriorated after this event Apneic spells occurred and in spite of the application of the Engström respirator the child died t[illegible] days later t the age of 21 days. During the last day there were bleedings from the gastro-intestinal tract

Roentgenologic find ngs. Roentgen exami nation of the chest carried out on the day of birth demonstrated wide-spread, regu larly distributed dense areas f reticulo granular ppearance in both lungs (Fig 4) The main bronchi and some of the larger subdivisions were clearly seen against the contrasting dense parenchyma. The pattern as characteristic of the hyaline membrane syndrome A moderate regression of the telectases was noted on the fourth day Confluent patchy infiltrates ppeared after 18 days and were considered t be secondary to aspiration.

The clinical diagnoses were immaturity – idiopathic respiratory distress syndrome in the neonatal period + pneumonia (aspira tion).

Gross findings at autopsy Both lungs were consolidated with foci of bronchopneumonia. The lungs weighed 30 and 35 g, respect vely (normal combined weight 44 ± 13 g) There was purulent material in the trachea and the main bronchi. The mucosa of the distal part of the esophagus was partly ulcerated. The remainder of the gastro-intestinal tract was normal. There were no cardiovascular ab normalities. The ductus arteriosus was pa tent. The other organs, including the brain, showed nothing remarkable

Bacteriologic find ngs Pseudomonas aeru ginosa was cultured from the lungs.

Histologic findings in lung specimens. (The sections were examined with the following stains Hematoxylin-eosin, v Gieson, Hei denhain Azan and Laidlaw reticulum stain.) Foci of bronchopneumonia were pre sent in both lungs. In many alveoli amor phous material was found indicating aspira

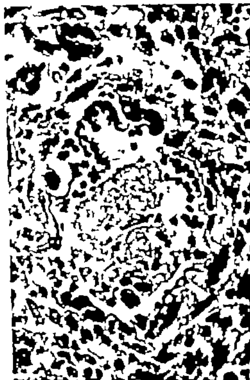


Fig 5. Thickened, partly hyalinized al eolar wall. Hyperchromatic nucl i in the al eolar epithelium. Amorphous material and some erythrocytes in the lumen. Premature infant (birth weight 1900 g), aged 21 day Id opathic respiratory distress syndrome in the neonata l period. (Case) Hematoxyl eosin 1[illegible]

tion. There were no hyaline membranes Man alveolar walls were t ickened and hyalinized in the same fashion as in Case 1 the nuclei of the al eola epithelium were h perchromatic and many [illegible] were observed in the interstitium. I this case in contrast t Case 1 there was [illegible] of col lagen well as reticulin [illegible] the thicke ned al eolar walls (Figs. 5

Case 3

Cl ical history This [illegible] a prema turely born male infant (birth weight 1320 g) the first hild of a [illegible] woman. On account [illegible] symptoms ppendectomy [illegible] performed



Fig 6. Thickened al veolar wall, containing f broblast and excess of collagen f bers (arrow). (Case ..) v Gieson 420.

the sixth month of pregnancy The appendix, however was normal, and the clinical symptoms were attributed to pancreatitis. After a pregnancy of .5 weeks the child was born in spontaneous normal vertex deli ery Within a few minutes after birth there was moderate respiratory distress (Silverman score 5) and slight cyanosis. After admini stration of 35 „ oxygen in the inspired air the cyanosis disappeared. During the first few lays of life the dyspnea gradually improved but the respiration was irregular and mainly of the Cheyne-Stokes type. Apneic spells occurred repeatedly and seemed to be provoked by oral feeding Therefore the in fant was gi en intra venous nutrition. During longer periods of apnea artificial ventilation with intermittent positive pressure had to be performed on one occasion for a period of 30 min ites

The general condition of the infant gradu ally deteriorated During the last days of lif cerebral symptoms were prominent: con ulsions occurred, there were muscular hypotonia and few spontaneous movements. The respiration rat decreased to °0-30/min. Comparatively long periods of apnea (<10 min.) supervened frequently The body weight had decreased to 1010 g when the infant died at the ge of 24 days.



Fig. 7 Detail from the same alveolar wall as represented in Fig 6. The collagen fibers are clearly sible (Case) Gieson 1320.

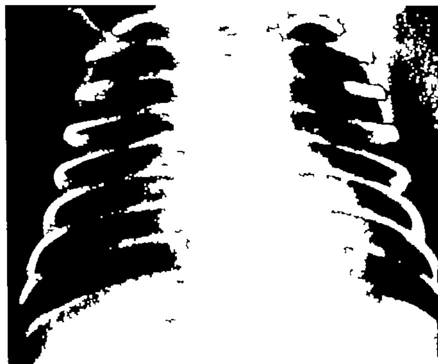


Fig 8. Case 3, three hours after birth. Dense areas of reticulo-granular appearance are scattered centrally and basally The air filled bronchi are well defined.

Roentgenologic findings. Roentgen examination of the chest performed three hours after birth showed areas of atelectasis of reticulo-granular appearance in both lungs. The central and basal portions of the lungs were predominantly involved The air filled bronchi were outlined against the surrounding dense parenchyma (Fig 8). The appearance of the lungs was essentially unchanged during the following five days. Progression was later observed. The abnormal pattern of the lungs and the sequence of findings were considered to be consistent with the hyaline membrane syndrome

The clinical diagnoses were immaturity + idiopathic respiratory distress syndrome in the neonatal period + convulsions, probably secondary to hypoxic cerebral damage

Gross findings at autopsy In both lungs the parenchyma was partly emphysematous, partly consolidated and there were foci of bronchopneumonia. The combined lung weight was 38 g (normal 30 ± 9 g) There were many petecchiae in the pleurae and the pericardium. The cardiovascular system was otherwise normal. The ductus arteriosus was closed Wide-spread hemorrhage in the leptomeninges was observed and there was a recent thrombus of the superior sagittal sinus. No macroscopic evidence of damage to the brain parenchyma could be recognized.

Bacteriologic findings Pseudomonas aeruginosa was cultured from the lungs

Histologic findings lung specimens. (The sections were examined with the following stains: Hematoxylin-eosin, Gieson, Heidenhain Azan and Lendrum reticulum stain.) In both lungs there were foci of bronchopneumonia and intraalveolar hemorrhage Many thickened alveolar walls of the same type as in Case 1 and were observed. In these alveolar walls fibroblasts and excess



Fig. 9 a. Case 4. One hour after birth. Small atelectases re diffusely distributed throughout both lungs.

of collagen a well as reticulin fibers could be recognized. There was no evidence of hyaline membranes.

Case 4

Cl ical history. This concerns a prematurely born male infant (birth weight 1990 g), second child of a healthy 23-year-old woman. Following an estimated gestational period of 38 weeks the child was delivered by caesarean section, because of profuse bleeding from a placenta previa. At birth the infant was pale asphyctic without spontaneous breathing. Resuscitation by int bation and artificial ventilation was immediately performed. Four minutes after birth spontaneous breathing occurred. Intercostal retractions were present from the first breath and there was generalized cyanosis. At three hours there was severe respiratory distress (Silverman score 9) and despite the administration of 80% oxygen in the inspired air cyanosis persisted. At that time a permanent catheter was inserted into one umbilical artery. Blood-gas analysis revealed an oxygen saturation of 30%, P_{aCO_2} of 75 mm Hg and met bolic acidosis (−23 mEq/l BE). The respiratory frequency was 40/min. Five hours fter birth all spontaneous breathing disappeared and there was a cardiac arrest during few seconds. After successful resusci tion by external heart massage intubation and artificial respiration with positive pressure the Engström respirator was pplied. The setting of the respirator was adjusted according t results obtained from blood-gas analyses. At the age of 12 hours adequat ventilation could be maintained by administrating 100 oxygen under a positive pressure of 50 cm H_2O and a respiratory frequency of 20/min. The oxygen saturation was 90 %, P_{CO_2} 35 mm Hg and BE −5 mEq/l in blood t ken from the catheter in the umbilical rtery

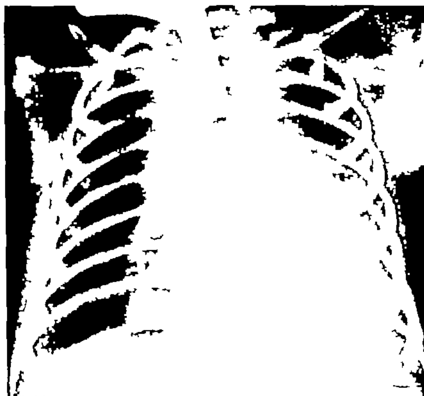


Fig. 9b. Case 4 One day of age following intubation and artificial respiration. The abnormal pattern is unchanged.

Two days after birth tracheostomy was performed.

The general condition slowly improved and from the third day the oxygen content in the inspired air and the degree of positive pressure could be gradually diminished. During the first four days of lif the hild was given intravenous nutrition, but thereafter oral feeding was started.

There was no blood incompat bility bet een mother and child. On the fourth day after birth, however serum bilirubin was 5 mg%. Since the respiratory and circulatory functions of the child were st ll impaired, no exchange transfusion was performed Audiometric studies were carried out from the 8th day of lif (Dr M Barr) howing reduced hearing No other clinical evidence of kernicterus was recognized. The serum bilirubin level gradually decreased following the fourth day

At the age of one month the artificial ventilation could be discontinued. Moderat intercostal retractions and prolonged expi rium persisted, however during the subsequent month. Decannulation wa not per formed until 2 months after birth

Because of the clinical and roentgenological findings (see below) thoracotom w per formed t the age f 56 days and small biopsy specimen was taken from the anterior segment of the right middle lobe for histologic examination. The postoperativ course was uneventful. When the child was sent home one month later there was still slight impairment of respiration evidenced by pro-



Fig. 10 Thickened alveolar wall with excess of fibroblasts and collagen fibers. Cellular amorphous material in the lumen. Biopsy specimen from premature infant (birth weight 1990 g) aged 8 weeks. Idiopathic respiratory distress syndrome in the neonatal period. (Case 4.) v. Gieson. 450.

longed expirium and rhonchi over both lungs.

Roentgenologic findings. Roentgen examination of the chest one hour after birth demonstrated findings that were compatible with those of the early stages of the hyaline membrane syndrome (Fig. 9a). In the examinations performed at ½ and 6 hours after birth the changes were more marked. The air filled bronchi were outlined against the surrounding dense parenchyma. On the following day after the patient had been intubated the abnormal pattern was still prominent in all parts of the lung (Fig. 9b). A slow but steady clearing followed. Emphysematous blebs and patchy infiltrates then developed in the right lung. Following a short period of enlargement they gradually diminished in size. After two months only small areas of telectasis remained. There were no abnormal changes seen in the left lung at this time.

The clinical diagnoses were immaturity + idiopathic respiratory distress syndrome in the neonatal period + hyperbilirubinemia + questionable kernicterus.

Histologic findings. (Biopsy specimen at 8 weeks of age from the right middle lobe. The sections were examined with the following stains: Hematoxylin-eosin, v. Gieson, Heidenhain's Azan and Lendrum's reticulum stain.) Parts of the lung tissue examined showed recent intraalveolar hemorrhage which was attributed to the trauma during operation. In some areas the alveolar walls presented the same type of hyaline-fibrous thickening as in Cases 1–3. In these alveolar



Fig. 11. Another part of the same biopsy specimen as respresented in Fig. 10. There is prominent thickening and fibrosis of the alveolar walls. (Case 4.) Gieson. 450.



Fig 12. Thickened alveolar walls with excess of reticulin fibers. (Case 4.) Landrum reticulum stain 10

walls there was excess of collagen as well as reticulin fibers (Fig 10–12) In some alveoli amorphous material containing a few histiocytes was found but there were no hyaline membranes.

Comment

All these four premature infants developed shortly after birth the characteristic clinical picture of idiopathic respiratory distress syndrome i.e grunting, nostril breathing severe intercostal retractions high respiratory frequency and generalized cyanosis [1 17] Roentgenograms f the chest showed areas of disseminated densities arranged in a reticulo-granular pattern all over the lungs. It is a well established fact that these linical and roentgenologic findings are associated with pulmonary hyaline membranes in pproximately 80% of the fatal cases expiring within the first postnatal week [.. 0 10 13, 14 16].

After the first few days of respiratory distress the four infants all improved gradually The ultimate cause of death in three of the cases was considered essentially unrelated to their initial disease It should be pointed out however that these three patients up to their last day of life as well as the fourth surviving patient t the time of lung biopsy still showed evidence of slight respiratory difficulties inspiratory retractions were present as well as a prolonged expirium

The histologic features in the lung tissue of the four cases were imilar Thickened alveolar walls were covered by hyperchromatic epithelium and showed increase of fibroblasts. In the three older cases (*1–56 days of age) xcess of collagen and reticulin fibers could be demonstrated in these walls. In th youngest there was excess only of reticulin fibers These features, apart from the develop-

ment of reticulin and collagen, resemble the proliferative and reparative phenomena occurring in connection with pulmonary hyaline membranes during the first postnatal week [3 15] Similar features have also been described in association with hyaline membranes in influenza pneumonia [10] The excess of reticulin and collagen fibers in the alveolar walls of our four cases is quite compatible with a late stage of such a process of reparation as referred to above

In the patient that expired on the 13th day hyaline membranes infiltrated by histiocytes could be demonstrated in the lungs In the other three surviving longer no hyaline membranes were present We believe that all these infants had pulmonary hyaline membranes in the immediate neonatal period as suggested by the clinical course but that the membranes in the older cases had disappeared or had partly been incorporated in the alveolar wall structure As mentioned before macrophage response including fragmentation and probably removal of the membranes been recognized at the age of 3–4 days in neonatal cases of this condition

Although the histologic features presented by these cases are compatible with a late stage of a reparative process in the alveolar walls probably secondary to a damage causing hyaline membranes in the immediate neonatal period, consideration must be given to the possibility that the treatment has influenced the development of the pulmonary lesions Experiments on adult rabbits and mice have shown that prolonged exposure to 70–100 oxygen causes alveolar damage and development of hyaline membranes [2 7]; fibrosis of alveolar walls, however has not been reported Newborn mice studied under similar experimental conditions have reacted differently emphysema of marked degree has been observed but no hyaline membranes [12]. The effects of prolonged exposure of the human neonatal lung to high oxygen levels have not as yet been analyzed Consequently no conclusions can be drawn concerning the possible effect of oxygen therapy in our four cases It cannot be ruled out however that this particular therapy though necessary for the maintenance of adequate ventilation might have influenced and possibly enhanced the structural alterations of the alveolar walls This applies particularly to Case 4 which received 100 oxygen during the initial phase of the respirator therapy

Even less is known about the effect of artificial ventilation with high positive pressure on the human neonatal lung Two of our cases (1 and 4) received prolonged respirator therapy one of these (Case 4) with high positive pressure In Case artificial ventilation was administered only during the last two days of life In Case 3 the respirator was never applied. Since the histologic features are similar in all cases the artificial ventilation *per se* can hardly have been a significant factor in the development of the lesions in the alveolar walls

In Case 4 emphysematous blebs developed during the respirator treatment. This complication can possibly be attributed to the high pressure ventilation Another point of interest concerning Case 4 is that clinical and roentgenologic evidence of pulmonary disease still persisted at the age of 2 months This is not the rule in uncomplicated cases of idiopathic respiratory distress syndrome in

the neonatal period. Other pulmonary le sions may have been present in this case though not detectable in the small biopsy specimen that was examined histologi cally It has to be emphasized that this biopsy specimen was obtained from the periphery of the lung

The alveolar lesions here described resemble those of infantile lobar emphysema [4] as well as those of the Hamman Rich syndrome [8]. There is still no evidence however of an etiologic relationship be tween severe respiratory distress in the neonatal period and the early phases of these two pathogenetically obscure pul monary disorders.

Summary

Four neonatal cases of idiopathic respi ratory distress syndrome suggesting pul monary hyaline membranes are presented Three of the infants died at the age of 13–23 days. The fourth survived and in this case a lung biopsy was performed at th age of 8 weeks. The histologic features in the lung tissue of all four cases were similar and consisted of thickened alveolar w lls showing increase of fibroblasts and excess of reticulin or collagen fibers. These findings are consistent with such a process of repair as has been observed in the first postnatal week in cases of pulmonary hyaline membranes. Hyaline membranes infiltrated by histiocytes were still present in the case that expired at the age of 13 days, but not in the three cases surviving longer Prolonged respirator therapy was performed in two cases, in one of these with high positive pressure All cases re ceived supplemental oxygen, one case 100 % oxygen during the initial phase of the disease The possible effect of this therapy on the development of the lesions in the alveolar walls is discussed.

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Studies of Urinary Tract Infections in Infancy and Childhood

III Quantitative Estimation of Cellular Excretion in Unselected Neonates

by KNUT LINCOLN and JAN WINBERG

Introduction

The diagnosis of urinary tract infections mainly rests upon the demonstration of a pathologic bacteriuria and leucocyturia. Accuracy in diagnosis thus requires a definition as clear as possible of the normal limits of urinary cellular and bacterial excretion. As a basis for the diagnosis of neonatal infections bacterial and cellular excretion have been established in about 600 unselected newborns. A report of the bacteriological part of the study has been published earlier [5] and demonstrated that accepted adult and childhood ranges for bacterial excretion were not applicable in neonates unless special cleaning procedures were applied. In this paper the urinary cellular excretion pattern will be reported. The results may to a certain extent explain the conflicting findings of earlier authors [1–4] dealing with neonates.

Material and Methods

The material has been presented in detail in a previous paper [5]. The urine specimen examined in this study are fewer than in the former one. This is due to circumstances working at random, and not the consequence of selection. The handling of the urine and different procedures for cleaning of the genital tract before collection of urine were also reported earlier. Since the count of the different types of cells were not appreciably influenced by different methods of genital cleaning all urine specimens, whether obtained after ordinary cleaning of the genital tract or after irrigation of praepuce or vulvar region, are presented together in the figures.

The cells in one cubic millimetre of uncentrifuged and unstained urine were counted in a Fuchs-Rosenthal counting chamber using the highest dry objective (× 40). In some instances—see below—the urine was stained with the Sternheimer Malbin stain [9] or with methylene blue. Gram-stained smears of urine or urinary sediment were also examined in these instances.

In the urine specimens mainly three different types of cells were found: leucocytes, squamous epithelial cells and non-squamous epithelial cells.

The shape of the non-squamous epithelial cells usually distinguished them from the leucocytes, although the size of these two types of cells was about equal (Fig. 1).

The classification of some oval cells was, however difficult. Cells with smooth,

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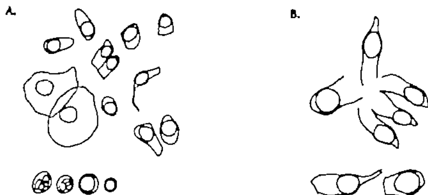


Fig 1 Line drawings after colour microphotographs of Papanicolaou-stained alcohol-ether fixed smears of urinary sediment from a 6-day-old boy *A* Two squamous epithelial cells (transitional cell) and numerous non-squamous epithelial cells. Not polymorphism, longation, angula ppearance and similarity t cylindrical or cuboid epithelial cells. One dubious cell to the right f the squamous cells. Bottom row: four leucocytes drawn t scale *B* The cells sometimes appear in clusters.

rounded contour were usually classified as leucocytes while cells with a more angular shape were characterized as non-squamous epithelial cells This judgement was some times subtle but since such d bious cell were relatively uncommon misinterpret tions probably ha e not ppreciably in fluenced the result of the cell counts.

In most of these specimens the lassifica tion w facilitated by the above mentioned staining procedures which perm tted a liable separation of the polymorph on iclea leucocy tes

Results

The results of the study are presented in Fig. * 3 and 4 Each figure shows the excretion of one type of cells and compares males and females

Leucocytes (Fig 2)

In both boys and girls most urine specimens contained between 0 and 9 leucocytes per mm of uncentrifuged urine. It is, however obvious that in the girls there are more specimens with high counts than in the boys. Thus 51 specimens out of 404 among the girls contained more than 25 cells per mm³ of uncentrifuged urine and 10 out of 404 more than 50 (Table 1) Among the boys corresponding figures were 7/344 and 2/344 If those samples are excluded which contained at the same time large amounts of coliform bacteria. The excretion during the first four days may be a little higher than that during the following 5–15 days

In male urine specimens with more than 50 leucocytes there were usually at the same time a high count of coliform bacteria. In Fig 2 such specimens are represented by black dots. Probably there was a urinary tract infection in these patients —as was discussed in detail in the previous paper Among the girls with a leucocyturia amounting to 50/mm³ or more there was on the other hand not a single case with high count (>100 000 bact. per ml) of coliform bacteria. A certain association to high counts of not identified slow growing bacteria of vaginal flora type was, however found in these cases (Table 2) Thus in girls especially those with more than 50 leucocytes per mm³ of

LEUCOCYTES IN THE URINE OF 498 NEWBORNS

Number of cells per mm³ of urine

Day of life

3 Th

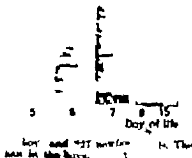
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Fig. — Leucocytes in the urine of 223 newborn boys and … Each symbol stands for a specimen containing … in an obs…

TABLE 1 Leucocytes …

Day of life	Boys: Frequency of specimens with …4 cells per mm³ uncentrifuged urine
1–4	4/39
5	0/15
6	0 136
7	/127
8–16	0 27
Total	7/344



boys and 237 newborn … The … in the boxes …

TABLE 2. *Result of anaerobic urine culture in patients with low and high counts of leucocytes.*

	Boys		Girls	
Number of leucocytes	< 25	—	< 25	> 50
Number of patients with < 100,000 bacteria per ml of urine	*0	—	*0	7
Number of patients with > 100,000 bacteria per ml of urine	0	—	3	6

Small grampositive and gramnegative rods.

urine prolonged incubation in closed plastic jars or under anaerobic conditions rather often disclosed growth of usually gramnegative and sometimes gramnegative and grampositive small rods, not unlike Haemophilus vaginalis as described by Edmunds [2, 3]. Such bacteria were not found when 25 specimens from *0 boys were cultured anaerobically (Table *).

"Glitter cells" or Sternheimer Malbin cells [8] were sometimes seen. Even in the absence of infection they could make up to 10 % of the leucocytes. They amounted to more than 50 % of the white cells in most instances of the coli infections.

Non-squamous epithelial cells (Fig. 3)

Boys excreted more of these cells than girls. The difference was statistically significant. With increasing age there was a diminution of the cells, which was statistically significant for the boys, but not for the girls (probably due to lower figures) (Table 3).

In boys a high count of non-squamous

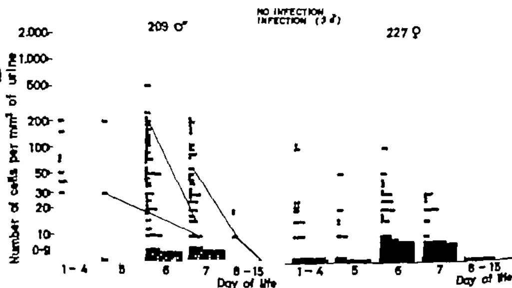


Fig. 3. Non-squamous epithelial cells in the urine of 209 newborn boys and 227 newborn girls. Symbols, confer Fig. 2. The figure suggests that these cells are appearing with higher frequency in the urine of the boys than in that of the girls. Excretion in the infected infants is not remarkably high.

TABLE 3 *Non-squamous epithelial cells in the urine of 436 neonates*

Day of life	Boys	Girls	Significance of difference between boys and girls
	Frequency of specimens with > 10 cells per mm³ of uncentrifuged urine		
1–4	22/30	17/46	$P < 0.001$
5	8/11	6/29	$P < 0.01$
6	68/131	31/146	$P < 0.001$
7	44/119	16/118	$P < 0.01$
8–15	7/20	3/15	—
Total	182/311	75/354	
	Significance of difference between consequent days		
1–5 / 6	$P < 0.05$	$P > 0.05$	
6 / 7	$P < 0.05$	$P > 0.1$	

epithelial cells was found much more often than a high count of leucocytes. In girls on the other side high counts of both types of cells were found with about equal frequency Epithelial cells amounting to 30 or more per mm were registered only in three of the 8 boys with a probable infection. They decreased with age in an expected way (black dots, Fig. 3) This decrease did not parallel the change in number of leucocytes in the urine

Squamous epithelial cells (Fig 4)

Fig 4 shows the excretion of these cells. It is obvious that boys excreted much less of these cells than did girls



Fig. 4 Squamous epithelial cells in the urine of 206 newborn boys and 227 newborn girls. The excretion is more marked in the girls than in the boys.

Comment

On the basis of this investigation we find it sound to suspect strongly a urine abnormality in the newborn and repeat the examination if there is a leucocyturia of more than 25 leucocytes per mm^3 of uncentrifuged urine in boys and more than 50 in girls, especially if the cells are of the granular motility type. These figures are a little more liberal than those given for older infants and children [8].

Figures for leucocyte excretion in newborns have earlier been given by James [4] and by Aas [1]. The former report presents very scanty figures which however probably are in accordance with ours. The figures given by Aas are not comparable to those of James and ours, since he did not separate leucocytes and non-squamous epithelial cells. In view of our findings of an inverted sex difference between leucocytes and non-squamous epithelial cells this may explain his statement that there is no difference between boys and girls as regards cellular excretion in newborns.

The leucocytes were to some extent of the "granular motility type" which are found in adults and children with renal parenchymal inflammations [9]. Their presence in the neonates may suggest a renal origin but since the frequency of those cells is related to the degree of urinary hypoosmolality [7, 9] their presence in noninfected cases simply may be a consequence of the low concentration of the urine during the neonatal period.

When boys excreted more than 25 leucocytes per mm^3 of uncentrifuged urine the leucocyturia was often marked and associated with a significant coliuria. The combination of marked coliuria and leucocyturia suggested a urinary tract infection as was discussed in the previous communication [5].

In girls the excretion of 50 cells or more was in none associated with marked coliuria. When culture was performed under anaerobic conditions it disclosed in several specimens an abundant growth of Haemophilus vaginalis-like bacteria. Such bacteria were found especially frequently in specimens with >50 leucocytes. Similar bacteria may in adults be associated with vaginal infections [7].

Thus a marked leucocyturia in newborn boys often seems to be caused by urinary tract infection, while in girls it may also result from a vaginal infection. Concerning the latter point, however, further studies are necessary before valid conclusions can be drawn.

The sex difference in excretion of non-squamous epithelial cells suggested by our findings is noticeable. We have no explanation for these findings. Lippman [6] refrains from assigning these cells to any special part of the urinary tract whereas Aas [1] classifies them as renal epithelial cells. The fact that the leucocyte excretion in our infected boys was of the same magnitude as the excretion of non-squamous epithelial cells in the noninfected cases suggests the value of making an effort to separate these cells, even if it may be difficult. Such a separation may be facilitated by various staining methods.

Summary

Urinary cellular excretion during the first week of life was studied in about 500 unselected newborns.

On the basis of the findings it seems

reasonable to suspect a urine abnormality when boys excrete more than 25 white cells and girls excrete more than 50 white cells per mm of an uncentrifuged urine. In boys the excretion of more than 25 white cells was usually associated with a significant colluria. Such patients probably had a urinary tract infection. In the urine of girls excreting more than 50 white cells Haemophilus vaginalis-like bacteria were frequently isolated in large amounts. The significance of this finding is uncertain.

Non-squamous epithelial cells were excreted in significantly larger amounts by boys than by girls. Excretion diminished with increasing age. The reason for this sex difference is obscure.

Squamous epithelial cells were present to a larger extent in the urine of girls than in that of boys.

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pressure in the right arm was 105/75, in the left 95/75, and in the legs 1?0/80. The ECG was repeatedly normal. X-ray of the heart showed a slightly increased volume (405 ml per square meter of body surface), but no definite abnormalities in configuration. Congenital heart disease was considered unlikely.

Lungs. The lungs were normal on X-ray.

Abdominal organs. The spleen, which had been palpable since birth, could still be felt 4 cm below the left costal margin, and the liver about cm below the right costal margin.

Genito-urinary tract. The penis was normal and the scrotal sac small but otherwise normal. Testes of prepubertal size and consistency could be palpated bilaterally at the external inguinal ring. The prostate was not palpable. In March 1961 a right inguinal herniorrhaphy was done. The right testis appeared normal, and a biopsy specimen was obtained.

Skeleton. X-ray films of the skull and extremities revealed no abnormalities.

Nervous system. Mentally the boy was retarded; he was regarded as an imbecile with a mental age of years as assessed by a Terman Merrill test and 2½ according to a Bühler Hetzer test. Neurological examination revealed no abnormalities. Ophthalmoscopic examination showed tortuous vessels in a pale retina. EEG showed an abnormally slow activity but no epileptogenic bursts.

Laboratory tests. Routine tests on blood and urine were repeatedly normal. The serum potassium, serum calcium, serum phosphorus, serum alkaline phosphatase, serum cholesterol, serum total lipids, and fasting blood glucose concentration were normal. The total serum protein was 7.0 g/100 ml with normal paper electrophoretic pattern. The urinary excretion of gonadotrophins was less than 6.5 MU/24 hours (normal). Paper chromatographic examination of the urine revealed no abnormal aminoacids.

Case M. B., a boy, was born June 2nd, 1956, to a 31-year-old mother and a 38-year-old father. A sister born in 1958 and both parents are healthy. No other member of the family has had signs or symptoms of the kind presented by the boy.

The boy was born at term after an uneventful pregnancy and delivery. His birth weight was 3050 g and his length at birth 49 cm. During the neonatal period he was found to be hoarse, and initially he had also difficulties in sucking. From an early age the mother noticed that he was shorter than other boys of his age. His psychomotor development was relatively normal; he was able to sit without support at the age of 7–8 months, he walked without support at 15 months of age, he started to talk single words at 1 months, and could speak short sentences at years of age. However, at the age of 5 his diction was still indistinct and he could not roll his r's. His mother has regarded him as being more easily exhausted by play than other boys of his age.

At the age of 3 years the boy was referred to the Department of Paediatrics at the County Hospital, Karlstad. He was short and peculiar-looking, with enlarged head, short neck, broad flat nose bridge, short, broad hands and feet, and very thin hair. A systolic murmur was heard. An air-encephalogram revealed slight central atrophy of both the lateral ventricles, and the basal cisterns were wider than normal. His mental development was regarded as normal.

At the age of 5 years the boy was admitted to the Department of Child Psychiatry in Karlstad, where a performance test showed IQ 103. In 1961 he was referred to the Department of Paediatrics, University Hospital, Uppsala, for further examination.

Examination at the age of 5½ years disclosed several anomalies (Fig. 2). The boy was only 99 cm tall (about 4 cm less than −2 SD) and weighed 16.5 kg (normal for the height). The skull circumference was 51 cm (normal). The hair showed several large whorls. The nose bridge was broad and flat. The neck was short, with moderately pronounced bilateral webbing. The external ears were low-set and showed slight anomalies. The palate was highly arched. There were no dental malformations. Hypertelorism, slight exophthalmos, and bilateral



Fig. 3. Case 1 Photomicrograph showing prepubertal testicular tissue with one large germ cell in the centre van Gieson, 350.

ptosis of the eyelids were present The voice was hoarse Laryngoscopy showed signs of laryngitis, but no malformations could be seen. The hands and the feet were short and broad. Typical cubitus and genu valgus were present on both sides.

Heart There were no signs of cardiac insufficiency t rest. The first sound was accentuated over the apex, where a grade I II, short presystolic murmur was heard. A harsh, grade III systolic murmur was loudest at the pex. A phonocardiogram showed this murmur to be of high frequency with a mid-systolic accentuation. The blood pressure was 105/90 in the right arm and 110/80 in the legs. The ECG showed frequent extra systoles from single focus within the left ventricle and P mitrale There were no definit signs of ventricular hypertrophy

On X-ray the heart olume was moderately increased (416 ml per square meter f body surface), with signs of left trial enlargement Cardiac catheterization showed slightly increased pressure in the left trium and a pressure gradient of 5 mm Hg between the left atrium and ventricle Other pressures were normal and no signs of intracardiac shunt were found Angiocardiography howed hypertrophy of the left ventricle with signs of systolic obstruction of the outflow tract The findings were interpreted as caused by idiopathic muscular subaortic stenosis.

Lungs The lungs were normal on X ray

Abdominal organs The liver was palpable at the right costal margin, but the spleen could not be felt

Ge ito-urinary tract. The penis was normal. The scrotal sac was small but otherwise normal, the testes could not be f lt The prostat was not palpable Intra enous urography showed no bnormalities. In September 1962 right-sided and in January 1963 left-sided orchidopexy was performed. The testes were situated very high in the bdomen, and were smaller than normal. A biopsy pecimen was taken from the right testis

Sk leton X ray films of the skull, the spine nd the extremities revealed nothing bnormal.

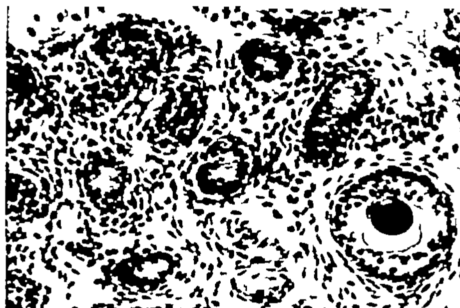


Fig 4 *Case 2* Photomicrograph showing prepubertal testicular tissue without identifiable germ cells. Not slightly increased cellularity and hyaline cast in one t bule. van Gieson, 230.

Nervous system. Mentally the boy seemed normal, and an intelligence test (Terman Merill) showed IQ 105 Neurological exa minat on revealed no bnormalities and the hearing was normal. EEG showed relatively slow basic rhythm, but the record was other ise normal.

Laboratory tests Repeated routine blood and urine test were normal. The serum cal cium, serum phosphorus, serum alkaline phosphatase serum holesterol and total serum l pid were normal. The serum protein bound iodine was 6 8 μg/100 ml. The urinary excretion of gonadotrophins was less than 6 MU/24 hours (normal) of 17 hydroxycorti costeroids (Norymberski) 9 mg/24 hours and of 17 ketostero ds, 0 6 mg/24 hours (normal)

Histology of Testis

The testicular b opsy specimens were fixed in formalin, and embedded in paraffin. Multiple 4-μ thick sections were cut and stained with haematoxylin-eosin, van Gie son s connective-tissue stain and by a routine PAS technique

Case 1 B H (Fig 3) There was no gross derangement of the testicular parenchyma, and no sclerosis The tubules were small and uniform in size. Their lumina were filled with irregularly stratified immature epithelium made up f rather small cells with compact, round or slightly oval nuclei. This epithelium contained vacuolated cells with predominantly basal orientation and larger lighter-coloured nuclei, representing primary spermatogonia. These cells occurred singly or in a few pairs and may have been rather scanty with regard to the boy ge The tubules were widely spaced in a loosely textured stroma containing small fibrocytoid cells. There were no differentiated Leydig cells. The tunica albuginea was normal.

Th find gs were compatible with pre pubertal testicular tissue without definite pathologic change

Case 2 M. B (Fig. 4) The general structure f the testicular tissue resembled that of Case 1 with small tubules of uniform size widely spaced in a rather cellular stroma of loose texture and without distinct Leydig cells. Thorough search in a multiple

Table 1 *Summary of chromosome analyses*

Origin of cultured cell		Chromosome counts 45	46	47	Total	Karyotype interpretation
Case I	Skin		24	1	25	Long Y chromosome
	Bone marrow	1	30	0	31	Long Y chromosome
	Testis	1	19		20	Long Y chromosome
Mother of Case I	Blood		32		32	Normal karyotype
Father of Case I	Blood	1	35		36	Long Y chromosome
Case II	Skin		15		15	Normal karyotype
	Blood		18		18	Normal karyotype
	Testis		6		6	Normal karyotype

sections failed to disclose any spermatogonia in the wholly undifferentiated tubular epithelium.

Compared with Case 1 there were two further differences of possible significance. A few tubules contained PAS-positive hyaline material (somewhat resembling corpora amylacea), which was partly surrounded by flattened epithelial cells—an unusual finding. The cells in the interstitial tissue were arranged rather irregularly and tended to be concentrated round the tubules. This tissue was slightly more fibrous than normal, with broader septuli, but no scar tissue was found.

The findings were compatible with prepuberal testicular tissue with germinal-cell aplasia.

Cytological Studies

Cytological studies of cultured cells from skin, bone marrow and testis were performed by the method of Böök *et al.* [4]. Chromosome studies of peripheral leucocytes were carried out by a modification of a method described by Moorhead *et al.* [19].

Case I B. H. Analysis of the chromosomes was based on a study of cell cultures derived from skin, bone marrow and testis.

Chromosome counts were made in a total of 76 apparently undamaged cells in

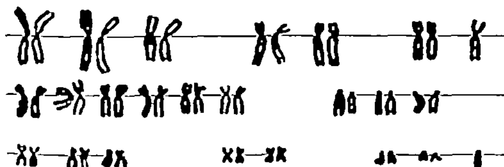


TABLE 2. *Chromosomal constitution in phenotypical males with webbed neck short stature and other developmental anomalies characteristic of the pterygium syndrome*

Authors	Age in yrs.	Testes on physical examination	Histology of biopsy specimens	Sex chromatin	Chromosome constitution
Fraccaro *et l* 1961	31	Undescended (intraabdominal)	Dysgenesis, germinal-cell plasia	Negative	Normal XY
Futterweit *et al.* 1961	12	Undescended (canalicular)	Dysgenetic t bules, germinal-cell hypoplasia, or plasia	Negative	Normal XY
Chu *et al.* 1961	1¾	Descended testes in normal scrotum	N t performed	Negative	Normal XY
Steiker *et al.* 1961	4/12 (Case)	Partly descended (inguinal canal)	Testes "immat ure in relation to the ge of 4½ months"	Negative	Normal XY
Steiker *et al.* 1961	13¾ (Case 4)	Descended, normal, prepubertal testes	Not performed	Not performed	Normal XY
Oikaw & Blizzard 1961	11 (Case 1)	Undescended (intraabdominal)	Dysgenesis germinal-cell aplasia	Positive	XX (One enlarged X)
Oikawa & Blizzard 1961	3½ (Case 3)	Descended testes in normal scrotum	Not performed	Negati	Normal XY
Laguens *et al* 1961	*9	Descended, small testes	Dysgenesis, germinal-cell dysplasia	Positive	XXY
ach *et al.* 1962	15 (Case 3)	Undescended (canalicular)	Dysgenetic tubules, germinal-cell plasia	Negative	Normal XY
de Gennes *et al* 1963	14½	Undescended (intraabdominal)	Dysgenesis, germinal-cell aplasia	Negative	? Normal XY
Authors 1964	10 (Case 1)	Partly descended (inguinal canal)	Normal pre-pubertal testi cular tissue	Negative	XY (Long Y)
Authors 1964	5½ (Case 2)	Undescended (intraabdominal)	Germinal-cell aplasia	Negati	Normal XY

mitotic metaphase The results of these analyses are summarised in Table 1

Detailed analyses of 11 cells with the aid of enlarged photomicrographs showed the chromosome number to be 46 with a normal male karyotype except that the Y chromosome was longer than usual (Fig. 5). The relative length of the patient s Y chromosome was compared with the relative lengths of the Y chromosomes of

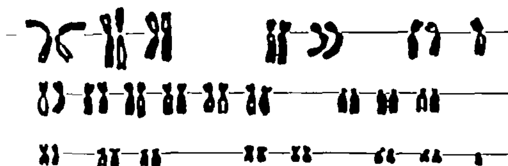


Fig. 6 Case 2 Paired chromosomes from testis showing normal male karyotype

6 normal men by measuring enlarged photomicrographs. All measurements were made by the same person

The mean relative length of the Y chromosome in 33 cells from 6 normal men was 18 7 (s.e. = 0 4, s d = 2 3). The variation in length of the Y chromosome among the 6 normal men was insignificant compared with the variation within each individual. The estimate of the mean relative length (in normal men) must therefore be considered adequate The mean relative length of the Y chromosome in 11 cells from the patient was 23 0 (s.e. = 0 2; s.d = 2.0) The relative length of the patient's Y chromosome was found to be increased by 23 % compared to the relative *length of the* Y *chromosome in the normal* material ($P<0\ 001$) No sex chromatin was found in the interphase nuclei of cultured skin cells from the patient

Chromosome studies of the parents were carried out on leucocyte cultures. Analyses of cells from the mother showed a normal chromosome number and karyotype The karyotype of the father was normal, but the Y chromosome was about the same length as that found in the patient. The mean relative length of the Y chromosome in 4 cells from the father was 23.1 which was found to be 24 % greater than that in the normal material ($P<0\ 001$)

Case 2 M. B Analysis of the chromosomes was based on a study of cell cultures derived from skin blood and testis. Chromosome counts were made in a total of 39 apparently undamaged cells in mitotic metaphase The results of these analyses are summarized in Table 1

Detailed analyses of 10 cells with the aid of enlarged photomicrographs showed *the chromosome number to be 46 with a* normal male karyotype The Y chromosome was of normal size (Fig 6) No sex chromatin was found in the interphase nuclei of cultured skin and testicular cells from the patient.

The *relative length* is the length of the chromosome relative to the total length of haploid set containing the X chromosome times 1000

Discussion

Both boys described showed multiple *somatic anomalies* characteristic of the

pterygium syndrome Both were short in stature and had a short webbed neck, hypertelorism slight exophthalmos bilateral ptosis of the eyelids a broad, flat nose bridge low-set anomalous external ears a high arched palate short broad hands and a small scrotum Other deviations from the normal were also noted In Case 1 there was mental retardation convulsions, and splenomegaly and in Case 2 hoarseness, cubitus valgus idiopathic myocardial hypertrophy and undescended testes. Above all, the boys differed in mental development which was normal in Case 2

In establishing to which sub-group of the pterygium syndrome in the male a patient is to be placed, the *histological appearance* of the testis seems to be decisive In Case 1 the testicular tissue was found to be of normal prepubertal type whereas in Case 2, in which the testes were not descended germinal cell aplasia was present The microscopical findings thus indicate the diagnosis of "Ullrich's syndrome in the male" in Case 1 while Case 2 is probably best classified as "Turner's syndrome in the male" These diagnoses are not to be regarded as definitely settled however as the present findings refer to boys of prepubertal ages, and therefore do not permit a prediction of the final, postpubertal testicular maturation.

The *chromosomal constitution* in male patients with the pterygium syndrome has only been studied in a very few cases [6 11 12 13 14 16 21 24 25], the pertinent data of which are collected in Table 2. With two exceptions these cases were found to be sex-chromatin negative and to have a normal male XY karyotype One exception [21] concerned an 11 year old child with undescended testes germinal-cell aplasia positive sex chromatin, and abnormal female karyotype with one normal and one enlarged X chromosome. The other exception [16] was a 29-year-old man with 47 chromosomes and the XXY karyotype of Klinefelters syndrome but lacking the histological testicular changes characterizing the syndrome Both of our patients were sex-chromatin negative Case 2 showed a normal chromosome number with a normal male karyotype. Case 1 also had a normal chromosome number but the Y chromosome was unusually long. This has not previously been reported in patients with the pterygium syndrome. The finding prompts a discussion concerning the importance of the long Y chromosome with regard to the developmental anomalies in this case This is of special interest, as Novakowski & Lenz [20] have drawn parallels with the XO situation in female cases, and claiming that mutation of the Y chromosome might produce part of the effect of absence of the whole chromosome

It is generally agreed that the length of the human Y chromosome varies not only in different cells from the same individual but also in different individuals. Variation in length of the Y chromosome may reflect differences in chromatin content in chromosomal behaviour or in both

An unusually long Y chromosome has been reported in an apparently normal male [2]; in a male mongol (trisomic for number 21) his normal brother and two distant relatives [3]; in a patient with Marfan's syndrome [15]; in two males with oligospermia [28], in a normal father and a paternal grandfather of a mongoloid

girl [18] and in a male infant, with multiple malformations, and his father normal in phenotype [28].

Studies with tritiated thymidin show that the Y chromosome of man replicates late during the period of DNA synthesis. This finding is compatible with the heterochromatic nature of the Y chromosome [1]. As duplications of heterochromatic segments are thought to have far less harmful effects than duplications of euchromatic segments of the same size [23] it would not be surprising to find occasional phenotypically normal individuals with an enlarged Y chromosome due to segmental duplication. The family of Case 1 may fall into this category and if so, the long Y chromosome of the patient has probably not contributed to his developmental anomalies.

The increased length of the Y chromosome might also be the result of a reciprocal translocation. The father could then have a balanced translocation between the Y chromosome and another chromosome, which translocation would have originated in the germ cells of an earlier generation. The other chromosome involved in the translocation ought then to be slightly shorter than normal. A small deficiency of one of the larger chromosomes might be undetectable. If the patient had a Y chromosome with a translocated fragment and was chromosomally unbalanced, he might have developed abnormally.

The usual finding of a normal chromosome constitution in the pterygium syndrome in the male brings up the question of the connexion between chromosomal aberration and developmental anomalies. Suffice it here to point out the interesting finding of the same developmental anomalies (the pterygium syndrome) both in cases with definite, but varying chromosome aberrations (Turner's syndrome in the female and some cases of Turner's syndrome in the male) and in cases with a normal chromosome constitution (Ullrich's syndrome in the female and in the male and most cases of Turner's syndrome in the male). It seems wise to warn against routinely attributing developmental anomalies to simultaneously occurring gross chromosome abnormalities. Obviously the same developmental abnormalities can appear both in cases with and in cases without apparent chromosomal aberrations.

Summary

Two boys with the pterygium syndrome are described. The first case showed a normal prepubertal testicular histology and an unusually long Y chromosome. The other had no germ cells and showed testicular dysgenesis, but had a normal karyotype. The first case is considered to represent Ullrich's syndrome in the male, while the other probably represents Turner's syndrome.

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CASE REPORT

Marked Elevation of Beta 2M Globulin in a Patient with Interstitial Plasma Cell Pneumonia

by K. KOUVALAINEN L. HJELT and O WASZ-HÖCKERT

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Interstitial plasma cell pneumonia occurs mainly in infants and especially in prematurely born infants. The disease is usually manifested between the first and fourth month of life and among institutional children it often occurs as small endemias [1 3 10 18]. The regular finding of Pneumocystis carinii in the lungs in histological sections points to the etiological role played by the organism [14]. In experimental animals Pneumocystis carinii is known to produce a disease which in many respects resembles the interstitial plasma cell pneumonia in children [21 23]

The occurrence of th disease especially in prematurely born infants aged 1–4 months points to a specially lowered resistance in this age group It is known that gammaglobulin values fall soon after birth, and thus prematures in the above mentioned age group show a gamma globulin level which is sometimes almost as low as that seen in agammaglobulinemia [8, 11] It is possible that this physiological impaired capacity to form gamma globulin is responsible for the lowered resistance against Pneumocystis carinii This hypothesis is supported by the fact that interstitial plasma cell pneumonia also occurs in older agammaglobulinemic patients [2 4 5 6 7 9 12, 13 15 16] On the other hand the therapeutic use of gammaglobulin in interstitial plasma cell pneumonia obviously does not improve or change the clinical symptoms [2, 6 12 16] Patients suffering from interstitial plasma cell pneumonia form antibodies against Pneumocystis carinii which can be demonstrated by the complement fixation test [17 22], but the existence of these antibodies in the commercial gammaglobulin is questionable

In the following a patient with interstitial plasma cell pneumonia showing marked elevation of beta 2M globulin is described. The immunoglobulins of 8 infants treated in the same institutional unit s th patient were also studied. The object was, firstly to see if elevated beta 2M globulin levels occurred in these infants as a possible sign of latent Pneumocystis carinii infections, and, secondly to detect the hypo- or agammaglobulinemic cases in order to give them prophylactic gamma globulin therapy



Fig. 1. Chest X ra showing diffuse infiltrations in both lungs nd small interstitial emphysema bubbles.

Ca e Report

Cl nical course and f nd ngs The patient (male) wa the first child of the family The pregnancy and del very were uneventful. The birthweight wa 3900 g The mother had tuberculosis of the lungs and the child was taken from the mother in the maternity hospital and treated in an institution for such infants. BCG vaccination was performed in the maternity hospital. The child was said to be limping a little but was otherwise normal. H did not gain weight normally in spite of an adequat food intake

At the age f 2 months and 4 days respira tory difficulties occurred and the child was brought to the hospit l H wa in bad condition. The weight was 4340 g, respiration fast. Moderat sternal retraction during inspiration was seen. In both lungs a few moist rales were heard. The heart sounds were normal, the nervous system and gastrointestinal tract symptomfree The temperature varied between 36.4 and 37.1°C The Hb was 13.6 g %. The leucocytes ranged from 10,800 with a normal differential count.

Chest X ray showed diffuse infiltrates in both lungs. Later on small interstitial emphysematous bubbles were seen (Fig 1). The serum protein pattern shown by paper lec trophoresis was as follows: total protein 5, albumin 3.1 alfa$_1$-globulin 0.2 alfa$_2$-globulin 1.0 bet globulin 0.7 and gammaglobulin 0.7 g/100 ml In the immunoelectrophoresis, done in the usual way using horse antihuman serum from the Pasteur Institute (No. 306) as antiserum, the gamma line was normal, the beta-2A hardly visible but the beta-2M line was very sharply accentuated (Fig.). The level of bet 2M globulin in the serum must have been, according t our experience about 5 times higher than in normal adult serum. The next day the patient was in very poor condition. The respiratory rate increased to 80–100/min. The skin was grayish. The temperature was not elevated. Bacterial culture from the nose showed Staphylococcus aureus, because of which the penicillin-streptomycin treatment started originally was changed to chloramphenicol and hcopenin. The child developed signs of cardiac insufficiency for which he was given digitalis. H remained in poor condition and died two weeks after the onset of the symptoms.

Autopsy The heart gastrointestinal tract, and central nervous and genito-urinary systems were without changes. The trachea

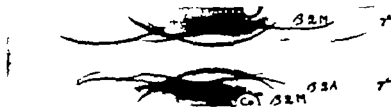


Fig 2. Immunoelectrophoresis of the patient nd control immunoelectrophoresis (Co). Not the marked accentuation of the beta-2M line and the gamma line, which is within normal limits.

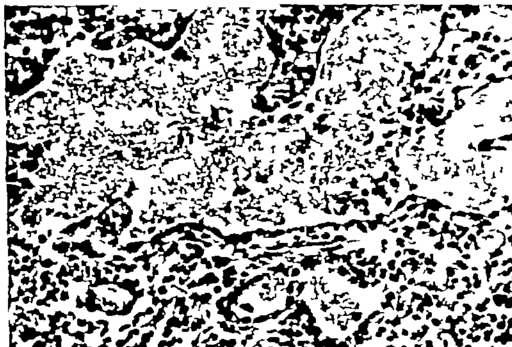


Fig. 2. Microscopic picture of the lungs. Note the accumulation of mononuclear cells in the thickened interalveolar walls and the foamy substance containing tiny spots in the alveoli (H.E., 400).

contained mucopurulent exudate. The left lung sank in water the right just floated. The visceral pleurae were red. The lungs were of varying consistency mostly rubberlike The cut surface was red and moderately consolidated. There was very little air and typical interstitial emphysema. The liver eighed 180 g and was congested.

Microscopically the lungs showed areas in hich the alveoli were filled with a foamy honey-comb like mass, in which tiny dark spots were seen. Numerous desquamated epithelial cells were present in some alveoli. The alveolar walls were thickened and showed marked plasma cell infiltration (Fig 3) There was congestion and patchy hemorrhages in the alveolar walls. The liver spleen and adrenals showed venous congestion, but ere otherwise normal. The kidneys were mature and normal apart from a few calcium containing calculi seen in few tubules in the left kidney The number of thymocytes in the cortical layer of the thymus was decreased. Hassall corpuscles were normal.

Of the eight infants who were exposed to Pneumocystis carinii infection by the pa tient described above, three showed hypogammaglobulinemia. In two of these three cases the gammaglobulin level, however was estimated t be higher than 0.3 g/100 ml, and prophylactic gammaglobulin treatment was given to one of the infants only The bet 2A and beta 2M lines were normal for that age. During the observation time of six months no manifest symptoms of Pneumocystis carinii infection was seen in the exposed infants.

Discussion

A marked rise of the beta 2M globulin in the described case with plasma cell pneumonia is beyond question. The immunoglobulins in interstitial plasma cell pneumonia have been studied extensively in the last few years, but an elevated beta 2M fraction in association with a normal

gammaglobulin level has not been described before. The patient described by Hong *et al* [12] with primary agammaglobulinemia exhibited a high beta 2M fraction. This however was elevated already before the onset of Pneumocystis pneumonia. Beta-2M macroglobulinemia is known to appear in the antibody deficiency syndrome as shown by Rosen *et al.* [20]. The patient presented by Marshall *et al* [16] probably primarily also had Rosen's type of agammaglobulinemia. In the patient presented here agammaglobulinemia is excluded, since the electrophoretically and immunoelectrophoretically *demonstrated gammaglobulin was in the* normal limits.

An isolated distinct accentuation of beta 2M is not normally seen but it is known that newborn infants can react against bacterial infections by forming antibodies which belong to the beta 2M fraction [8]. This patient did not have a manifest bacterial infection at autopsy in spite of the positive nose culture for Staphylococcus aureus. The ASTA titers unately were not studied.

While it is thought that beta 2M globulin is formed in lymphocytoid plasma cells [10], accentuation in *interstitial plasma* cell pneumonia could be expected to arise from massive infiltration of the alveolar septa with plasma cells. Agammaglobulinemic patients which exhibit normal beta-2M levels do not show plasma cells in the lungs in spite of a pneumocystic infection [5]. Accordingly accentuation of beta-2M could be a consequence of the highly increased number of plasma cells. Whether in this disease the cells form specific antibodies against Pneumocystis carinii cannot be decided without complementary studies.

Since interstitial plasma cell pneumonia is a special danger for children in institutional care with hypo- or agammaglobulinemia, it seems important to study the immunoglobulin status of exposed children. During the incubation time gammaglobulin therapy for hypogammaglobulinemic children for 1-2 months might be of importance. The commercial gammaglobulins contain very small amounts or none of the beta 2M fraction which might be important in this type of pneumonia. The poor results with gamma globulin in cases of manifest disease [2, 6, 12, 16] might also be due to lack of the *beta-2M fraction in the preparation used.* Specific gammaglobulin preparations with a high level of antibodies against the Pneumocystis carinii would possibly be a reasonable trial in the therapy and prophylaxis of interstitial plasma cell pneumonia.

Summary

A two-month-old male infant suffering from typical interstitial plasma cell pneumonia showed a marked elevation of beta-2M globulin in association with a normal gamma globulin level. The significance of this *finding was thought to be due to the* massive plasma cell infiltrations in the lungs. Possible value of gammaglobulin preparations in prophylaxis and treatment of the disease has been discussed.

Addendum

While this paper has been in print the writers have seen an other case of interstitial plasma cell pneumonia in the same institution as the patient described above. Also this patient showed a strong accentuation of beta 2M-globulin.

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PROGRESS IN PEDIATRICS

The Course of the Respiratory Distress Syndrome of Newborn Infants

As Indicated by Poor Stability of Pulmonary Expansion

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Until recently most investigators of the pathology of the respiratory distress syn drome (RDS) gave prominent considera- tion to the two pulmonary changes which could readily be ascertained at autopsy· atelectasis and hyaline membranes (HM) More recently another pulmonary factor was found to be regularly associated with the RDS namely poor stability of lung expansion (PS) caused by a deficiency in the peculiar surface activity of the lining ᴫ of the respiratory surfaces. Data from autopsy material pertaining to these fac- tors have now accumulated in sufficient numbers to suggest an interpretation of the pathogenesis and course of the pulmonary disturbance in the RDS which will be presented here Since we will be concerned with the incidence of HM and PS in rela- tion to each other and to birth weight and length of survival, the concepts currently associated with these two conditions will at first be briefly reviewed.

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Hyaline membranes A detailed review has been presented by Gregg & Bernstein [7]; only the highlights will be mentioned here For a long time the combination of HM and atelectasis was the only patho- logic finding in infants, mostly prema tures, with the RDS. It was surmised that the HM cause the persistence if not the development of secondary or resorption atelectasis The appearance of HM only after some time usually hours of air breathing seemed to correlate with the notion that the RDS develops following a period of normal respiration. On the other hand it has been pointed out that atelec tasis is remarkably uniform while the distribution of HM as seen at autopsy is spotty in many lungs and absent in some and this led to the conclusion that the membranes are an accessory of some other less conspicuous abnormality rather than the essential lesion. When it was recogni zed that the basic constituent of HM is fibrin it was obvious that transudation had to precede membrane formation yet this is not the usual pulmonary edema since the lungs are at no time "wet

either by auscultation during life or by examination at autopsy Evidence has been accumulating that the RDS does not develop in infants who were normal for a few hours after birth, but has its inception immediately following birth, probably pre ceding the formation of HM.

Poor stability of expansion Avery & Mead [1] and Gruenwald [8 9 10] observed that the pressure-volume curves of some neonatal lungs lack the usual degree of hysteresis. Poor stability of expansion is the result of air loss from these lungs occurring on deflation at relatively high pressure while specimens of normal infant lungs retain more than half their air con tent at deflation to 5 cm these abnormal lungs lose more than half their inspired volume at considerably higher pressures [10] and many of them are completely airless in the absence of transpulmonary pressure regardless of the maximum volume registered at higher pressures This is the state usually seen at autopsy The above mentioned authors found a good correlation of PS with the RDS or with HM. It is now established that normal hysteresis, and stability of expansion is the result of lowering of th surface ten sion along the alveolar walls specifically during expiration, produced by characteristic surfactant which is presumably elaborated by alveolar lining cells. A deficiency of this activity (we can only measure activity not the concentration of the active lipoprotein) results in PS Barrie [*] and Pattle and coworkers [18] have also correlated this surfactant deficiency with the RDS. Given an intact lung specimen, the stability of expansion can be determined by obtaining a pressure-volume curve [10] In the present study most lungs were inflated stepwise with pressures up to 60 cm of water and then deflated again in steps. Variations in deflation characteristics and therefore the stability of expansion are most significant and conspicuous at pressures below 10 cm An index was devised which is essentially a measure of the area below the deflation curve between 10, 5 and 0 cm of pressure [12]. This index ranges from 0 to 1.50 and when actual observations were plotted, they fell into one group below 0.71 and another above 0 80 Very few observed values were between 0 71 and 0.80, and it turned out that this provides a convenient border between good and poor stability Detailed data including most of the cases used here, were previously given [10]. In the present report only the two groups of good and poor stability will be contrasted, without re gard to individual values within each group

The consequences of PS for an infant s respiration are obvious. The first breath is unaffected However in contrast to the normal state in which a residual volume of air is rapidly established after birth these abnormal lungs lose their air content completely or nearly completely at expira tion. Each subsequent inspiration must therefore open up a collapsed lung again and again, and thus requires an inspiratory effort almost as great as that of the nor mal first breath. This increased effort is clinically obvious. If it is sufficiently strong it may at each inspiration open the lungs approximately to the extent that they would normally open at the first breath. As it weakens not even this can be accom plished any longer In premature infants who constitute a great majority of cases

of the RDS these difficulties are compounded by the tendency toward atelectasis of prematurity [10]. In this condition the bronchial tree, including the respiratory bronchioles, expands under moderate pressure while a much higher pressure is necessary to expand the alveoli. If inspiratory effort is vigorous the lungs may be fairly well expanded but will return to the state of atelectasis of prematurity if stability is poor. If the respiratory effort becomes inadequate after fatigue supervenes the state of atelectasis of prematurity may not be overcome and the infant is then limited to breathing with its respiratory bronchioles. This is not only inefficient, it also prevents the increase in lung volume which normally occurs after several hours of breathing [10].

Observations

Information was obtained from autopsy material by two lines of investigation, namely pressure volume curves of whole lungs and microscopic study of artificially expanded as well as intact tissue. In all 320 usable pressure volume curves were obtained. The present report is limited to 194 cases, namely those with a body weight at autopsy of 751 to 2500 g. These include the ones previously reported [10] and additional material obtained since then. In all instances sections of tissue not subjected to experimental expansion were examined microscopically, and in some cases also expanded tissue fixed at known pressure [11].

Poor stability and hyaline membranes. Fig. 1 summarizes the incidence of PS and HM in five age groups. The typical, full

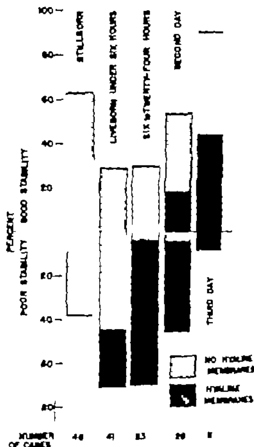


Fig. 1. Poor stability of expansion and hyaline membranes in infant lungs at autopsy. All bars are of the same length (100 %) and are divided according to stability (above and below dividing line) and microscopically recognized hyaline membranes (shading).

countered among infants dying between six and twenty four hours after birth. At this age the correlation of the two parameters studied is nearly perfect. In the entire group of 53 cases only 2 with HM had good stability just above the border line and two without HM had PS; these were seven hours old and might yet have developed membranes had they survived longer. Even if it were assumed that PS

the presence of membranes is in this age group a good pathologic-diagnostic indicator of the disease regardless of its significance in the pathogenesis.

Tracing the condition back to infants dying during the first six hours after birth, one finds the same incidence of PS but fewer HM. This is consistent with the possibility that the RDS is as prevalent among neonatal deaths during this period as later during the first day but has not yet led to the formation of HM in the majority of cases. If this were true PS but not HM would be a good indicator of the disease at autopsy in this age group

The state at birth is represented by findings in stillborn infants; in this group there are obviously no HM. In contrast to neonatal death death in utero is not caused or contributed to by PS since it occurred before respiration could have been attempted. It is therefore significant to find that 37 % of these infants had PS It is unlikely that this is representative of the entire population of newborn infants, and one is therefore led to the conclusion that there is some association of fetal death and PS or in other words, that some factor or factors causing fetal death also produce PS

When observations are continued beyond the first day there is again a departure from the good correlation of PS and HM In contrast to the first six hours, however death on the second day yields a significant number of lungs with HM and good stability but not the reverse situation. If by the end of the first day there is a nearly perfect correlation of PS and HM, it may be concluded that on the second day about one third of all lungs with HM and previously poor PS have now

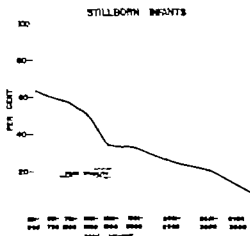


Fig _ Incidence of poor stability of expansion in the lungs of 100 non-macerated stillborn infants by birth weight groups.

quired good stability perhaps as a beginning of reversal of the basic abnormality of the RDS This trend continues on the third day when the majority of lungs with HM have good stability The present material does not include lungs with HM of infants older than three days.

Maturity factor The RDS predominantly affects premature infants. It has been suggested that persistence of a premature state f the lungs comparable to that normally existing in infants weighing less than 1°00 g is responsible for a deficiency in surfactant resulting in PS [1] The incidence of PS in relation to birth weight may best be studied in stillborn infants in whom it did not contribute to the fatal outcome In order to demonstrate the trend of incidence infants of all birth weight groups were included here in contrast to the limited group represented in Fig 1 Macerated stillborn infants were excluded from this study Fig 2 shows that 33 % of previable fetuses weigh

ing less than 500 g had good stability and an occasional infant weighing over 3000 g had poor stability. The distribution does not suggest an exclusive or even greatly predominant effect of prematurity, since both poor and good stability occur in all weight groups extending from far below the border of viability up to full maturity. As was indicated above, some stress factor of unknown nature predisposes to both fetal death and PS. A combination of this factor and immaturity can adequately account for the distribution seen in Fig. 2.

As was mentioned above, atelectasis of prematurity may compound the effects of PS. In mature lungs, PS and HM are found without the premature pattern of atelectasis, and HM may then be found in the partially expanded terminal air spaces as well as the respiratory bronchioles [11].

Discussion

The present observations confirm the preeminent significance of PS in the pathogenesis and manifestations of the RDS. On this basis the following tentative account of the course of the pulmonary abnormality in the RDS may be given.

Prior to birth an unknown form of stress affects the fetus; this form of stress may occur more frequently or severely in prematures, or may combine in prematures with a separate immaturity factor which enhances its effects. A few conditions are known to be prominently associated with this occurrence, such as maternal hemorrhage [5, 19] or diabetes [6]. Whether or not delivery by cesarean section has a similar effect is not decided. However, in many instances neither of these factors is involved. Whatever the cause, a variety of evidence [3, 5, 8, 15, 16, 17, 19, 20] suggests that the RDS develops immediately after birth and is presumably due to prenatal factors. Usher [21] has aptly summarized this as follows: "The syndrome probably originates in utero, for it is usually evident from the first breath." He has also made the point that difficult delivery in the obstetrical sense, which produces acute perinatal distress, is apparently not the same factor which produces or predisposes to the RDS. Now that we no longer depend for the pathologic diagnosis of the RDS on HM, which may require hours to develop, we can find evidence for the prenatal origin in the relatively high proportion of stillborn infants with PS [8].

The onset of respiration after birth should not be affected by PS. However, failure to establish a functional residual capacity of the usual magnitude, and collapse of the lungs recurring at each expiration, necessitate an excessive respiratory effort, as was mentioned above. A vicious circle ensues, and the more the fatigue and metabolic disturbances secondary to hypoxia increase, the less the infant is able to expand its terminal air spaces again and again on inspiration. Meanwhile a transudate furnishes the fibrin which forms the essential component of the HM. It has been suggested that abnormally high surface tension predisposes to transudation [2, 4].

It is suggested, though not conclusively proven by the present data, that on the second day the surfactant deficiency which produces PS begins to correct itself. There is probably considerable variation in the time at which this occurs. If at that time the infant is in sufficiently

good condition, it may now begin more nearly normal respiration and recover As part of this recovery the lung must in crease its total volume to the extent that this happens in other infants during the first day

The present material includes eleven infants who died on the second or third day with HM and good stability Search was made for anatomical evidence which might explain the fatal outcome in this group in spite of an opportunity for im proved respiration. One of these infants weighed less than 1000 g; seven others showed either rupture of lungs, or evidence of heart failure (fatty degeneration of the myocardium or greatly dilated left ven tricle) or intracranial hemorrhage of significant severity Only three infants in this group showed none of the above men tioned conditions In none of this material was any evidence available to indicate the degree of metabolic derangement which may have been present in addition to the anatomical factors. These observations confirm the suggestion that infants will survive the RDS when the stability of their expansion improves after the first day if they are not otherwise damaged. Efforts to support infants with the RDS metabolically are particularly important as long as we cannot prevent or treat the pulmonary lesion. We should make every effort to guide the infants over this self limited disease in the best possible condition in order to enable them to recover when the stability of their expansion returns to normal.

The present observations bear on the interpretation of autopsy findings in neonatal lungs In infants dying more than six or seven hours after birth, the presence of HM is probably the best diagnostic criterion of the RDS On the other hand, in infants dying earlier the demon stration of PS is more valuable During the latter part of the first day when most of the infant deaths with the RDS occur both criteria are equally reliable If an intact unfixed lung is available (while the other lung is being fixed and sectioned) a pressure-volume curve may be prepared with simple equipment and in a short time If only pieces of lung tissue are available they may be examined on a sur face balance with a variable area this re quires more elaborate equipment and more time Both methods yield comparable re sults [14]

Many pathologists have been puzzled by the airless state of the lungs of infants with the RDS even though respiration must have taken place for hours. It is now clear that in the absence of the usual stability of expansion the lung will expel all or most of the air contained in it when it is not exposed to transpulmonary pressure This is not resorption atelectasis, but a phenomenon which may be reproduced at will in post mortem specimens by arti ficial inflation and subsequent reduction of pressure Thus the airless state of these lungs at autopsy is no indication of the amount of air which they may have con tained during inspiration. One might, in fact use this airless state of a lung which is known to have contained air for con siderable periods of time as evidence of PS This has been tested against pressure volume curves in many specimens and has been found to be uniformly correct Obvi ously this criterion cannot be used in the lungs of infants who have not breathed at all or only for short period of time

While it is becoming apparent that PS can occur under conditions other than the RDS and at any age [13] the usual perinatal autopsy material contains only a very small number of instances of PS which cannot be accounted for by the RDS

Summary

A review of the findings in lung specimens from perinatal autopsies had led to conclusions which agree with the concept that poor stability of expansion caused by deficient activity of a specific surfactant is the basic lesion in the RDS

On this basis the following course of the pulmonary abnormality is suggested. The basic lesion originates before birth through the action of an unidentified form of stress, and more commonly in premature infants. Failure to establish a functional residual capacity and collapse at each expiration account for the gradually increasing difficulties of infants afflicted with this defect. Hyaline membranes develop after several hours. The defect of surfactant activity is self limited and reverses itself on the second or third day When this happened, infants recover unless they are irreversibly damaged by the RDS, or by other pathologic conditions.

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SUMMARIES OF SUPPLEMENTS

Mongolism in Newborns

A Clinical and Cytogenetic Study

by BERTIL HALL

(*Supplement 154*)

All newborn, suspected mongoloid infants in south Sweden during the period 1/7 1961–30/6 1962 were investigated clinically and cytogenetically Only those (38) suspected of mongolism who moreover had trisomy 21–22 were classified as mongoloid. This is the principal material of the investigation upon which the conclusions are mainly based. These children were follow up investigated at age one year The mongolism frequency calculated on the number of newborn infants, was about 1.5 ‰. The control material consists partly of 40 normal newborns investigated clinically and cytogenetically partly of recorded information concerning 207 newborn children. In addition, other pediatricians clinically investigated 20 newborn suspected mongoloid infants born during the period 1/7 1962–30/6 1963 according to a predetermined questionnaire They also investigated 46 normal newborn infants, according to the same questionnaire. The suspected mongoloids investigated by other pediatricians were also cytogenetically investigated. At x ray examination of the hands, adults were also investigated, i.e. 10 mongoloids, 9 oligophrenics of unknown genesis, and 29 normals.

Of the many signs of mongolism in newborns, the following 10 were chosen as cardinal signs: *a*) lack of Moro reflex; *b*) muscle hypotonia, *c*) flat facial profile *d*) oblique palpebral fissures *e*) dysplastic ear *f*) abundant neck skin, *g*) typical or atypical four finger line *h*) hyperflexibility *i*) dysplastic pelvis *j*) dysplastic middle phalanx dig V Two of the signs, lack of Moro reflex and muscle hypotonia are hypothetically combined with a depressed CNS function. Three cardinal signs can be localized to the head. flat facial profile, an easily observable sign that seems mainly due to os maxillare hypoplasia oblique palpebral fissures accepted as a good sign in older mongoloids and seemingly equally valuable in newborn mongoloids; and the dysplastic ear The ear morphology varies more in mongoloids than in normals but the *dysplastic ear is* usually easy to distinguish from the normal ear The comparison between the dysplastic ear and the normal ear in the newborns can be facilitated by the fact that the ears in newborns seldom protrude. The mongoloid ear is smaller has a more rounded form, and the helix has not the distinct morphology found in the normal ear The sixth cardinal sign, abundant neck

skin, is easy to appraise It represents also the general skin change at mongolism, i.e the pastmess and flaccidity signs found also in the palms of the hands. The seventh cardinal sign is the four-finger line typical or atypical In this work, only those were included among the atypical four finger line in whom an obvious fusing had occurred between the three-finger line and the five finger line. The eighth cardinal sign, hyperflexibility is a well-known sign of mongolism and seems to be as valuable in newborn mongoloids. The ninth and tenth signs are roentgenological. The roentgen examination of the pelvis showed that the *iliac index* is not suitable for use from a diagnostic point of view in newborns because mong other things, small turnings round the transverse axis produce great changes of the acetabular and ilium angles. The morphologic deviation in the mongoloid pelvis (ilium) which is usually very clear is more simple to appraise It has been pointed out that, because the mongoloids normally have a dorsally outwards curvature of the iliac crest, usually no bone process is visible on the place of spina iliaca posterior superior This additional sign is valuable because it is not affected by a moderate turning round the transverse axis. The x ray examination of the hand skeleton shows that the middle phalanx dig. V in the newborn mongoloids is usually dysplastic This dysplasia is probably rare in adult mongoloids This examination shows, moreover that it is usually easy to judge whether the middle phalanx dig V deviates morphologically but that it is hazardous to judge whether a morphologically normal middle phalanx is short or not. Of the other signs that have not been chosen as cardinal, it can be mentioned that the newborn mongoloids were often pale, quiet, had a weak cry and lay in a characteristic manne with the legs abducted. Epicanthus is lightly pronounced in the newborn mongoloids whereas the inner eye angle lacks the distinct pointed morphology seen in normals The blunt inner eye angle is, as it were filled with small skin creases. Iris spots are a valuable sign of newborn mongoloids. The patellar reflexes are often weak in newborn mongoloids. The newborn mongoloids do not have a flat occiput but a rounder head shape than the normals. The abundant neck skin helps positively in giving the investigator the impression that the head shape is round. This view is based on the fact that the lateral roentgenograms did not show silhouettes clearly distinguishable in this respect from those of normals, and that a highly premature mongoloid, apart from a normal neck skin, had also a normally protruding occiput and thus not a round head shape The newborn mongoloids however have a smaller head circumference than normals with corresponding birth weight and it could also be proved that the fronto-occipital diameter in the newborn mongoloids is significantly shorter than in normals with corresponding birth weight The large fontanel in the newborn mongoloids seems somewhat bigger than in newborn normals, but the difference is not great enough to be of diagnostic value The pregnancy period is shorter for the mongoloids than for the normals. The mongoloids have, moreover a lower birth weight than can be explained by the curtailed pregnancy period. Where the mongoloids were full term (pregnancy period more than 40 weeks) there was no difference between

mongoloids and normals concerning birth weight. The skeletal age in the wrist is advanced in newborn mongoloids compared with newborn normals. Where six or more of the cardinal signs are positive and the picture also otherwise argues for the diagnosis mongolism, i.e. where also other positive signs occur a chromosome investigation, from a diagnostic point of view is not considered necessary.

The chromosome investigation does not give an absolute diagnosis. The diagnosis should be based upon the clinical investigation. A subject resembling mongolism, with trisomy 21–22 and with a normal set of chromosomes in a percentage of the cells (mosaicism) has been shown to have normal intelligence. One must thus take into account the risk that only the aberrant cells are observed at the cytogenetic routine investigation, especially as the frequency of normal cells can vary from tissue to tissue. A chromosome investigation of all uncertain cases, however should be carried out. No complicated arrangements for sending samples for cytogenetic investigations are required. Of the 43 suspected mongoloids investigated 1/7 1961–30/6 1962 38 proved to be trisomic for a chromosome in the group 21–22 whereas the other 5 had apparently normal chromosomes. The chromosomes were in most instances examined in two tissues, blood and skin. Of the five subjects with normal set of chromosomes, three had four of the cardinal signs and in addition two of these proved at age one year to be mentally retarded. These mongolism resembling subjects, with mental retardation but without signs of chromosome aberration, were called pseudomongoloids.

Fifteen of the 38 mongoloid infants died during the first year of life; all before age six months. Ten were autopsied, all of whom had cardiac malformation. In seven, ventricular septal defect occurred, and in one or two persistent ostium atrioventriculare commune. The last mentioned malformation is then not thought to be such a dominant heart malformation at mongolism as was earlier imagined. Of the five not autopsied two had an obvious systolic murmur. Where suspicion of cardiac malformation remained in the 23 surviving at age one year a fresh x ray examination of the heart and an electrocardiogram were made. After this examination, suspicion of heart malformation remained in 3/23 subjects.

All mongoloids follow up investigated at age one year showed signs of mental retardation in varying degrees. It would seem that most signs described in older mongoloids are already present in the newborn mongoloids. Mental retardation, however cannot be demonstrated, but it must be pointed out that certain signs indicate a depressed CNS function in the newborn mongoloid. The experiences gained at the investigation of the newborn material and of the age one year mongoloids do not suggest that it is much easier to diagnose when the mongoloid has reached age one year. In some cases, the reverse seems to apply.

The present investigation demonstrates a pronounced variation in the phenotype of newborn mongoloids. This agrees with the view of some authors concerning older mongoloids—a view which, however has not been altogether accepted in the literature.

Based upon a comparison of the embryonic development of the finger pha-

langes and the phalanx morphology in newborn mongoloids, a hypothesis is presented to explain some aberrations in mongoloids. This hypothesis, delayed embryonic development and various possible alternatives to the mechanism giving rise to this process are discussed

Respiratory Studies in Children XI Mechanics of Breathing Lung Volumes and Ventilatory Capacity in Asthmatic Children from Attack to Symptom Free Status

by INGA ENGSTRÖM

(*Supplement 155*)

Twenty-one asthmatic children between the ages of 6 and 17 years were studied, 11 boys and 10 girls Pulmonary flow resistance dynamic compliance static lung volumes and timed vital capacity were determined during an asthmatic attack and repeatedly during recovery until a symptom free status was attained.

During attack pulmonary flow resistance, functional residual capacity and residual volume were increased and dynamic compliance vital capacity forced expiratory one second volume and total lung capacity were decreased. A relationship was found between the degree of pulmonary function aberration and the intensity of attack as judged clinically The functional residual capacity at attack was influenced both by pulmonary flow resistance at attack and by the functional residual capacity at symptom free period The reduction of forced expiratory one second volume at attack was better correlated to the reduction of vital capacity than to the increase in pulmonary flow resistance. The reduction of dynamic compliance at attack was correlated to the reduction of vital capacity

The pulmonary flow resistance returned rapidly to normal values on recovery from attack. The static lung volume and the forced expiratory one second volume reversed at a slower rate continuing also when the pulmonary flow resistance was normal. At symptom free status the static lung volumes were increased, pulmonary flow resistance was usually normal, dynamic compliance was increased and the forced expiratory one second volume was still decreased Dynamic compliance and vital capacity had the same relationship as in healthy children through the different stages of disease. Forced expiratory one second volume and pulmonary flow resistance had the same relationship through the different stages of disease but considerably different from the relationship in healthy children insofar as forced expiratory volume always was lower than would be expected at the pulmonary

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flow resistance found. The relationship between functional residual capacity and pulmonary flow resistance at attack differed from the one at symptom free period. At attack the functional residual capacity increased in relation to the increase in pulmonary flow resistance. At symptom free or almost symptom free status the pulmonary flow resistance increased when functional residual capacity decreased.

The mechanism at attack, recovery and symptom free period was discussed. Other probabilities than bronchial obstruction for the maintenance of hyperventilation and ventilatory impairment during symptom free periods were suggested.

Nils Rosén von Rosenstein and His Textbook of Pædiatrics

edited by BO VAHLQUIST and ARVID WALLGREN

(Supplement 156)

Jubilee-volume celebrating the 200 years anniversary of the publication of Nils Rosén von Rosenstein's famous book "Underrättelser om Barnsjukdomar och deras Bote-Medel" in 1764. The volume had the following contents.

Preface by Torgny Segerstedt

The Nils Rosén von Rosenstein Medal by John Lind

Nils Rosén von Rosenstein, a short Biography by Arvid Wallgren

The Diseases of Children and their Remedies by Bo Vahlquist

Nils Rosén von Rosenstein and Iatromechanics by Anna Lena Pehrsson

Correspondence between Nils Rosén von Rosenstein and Albrecht von Haller by Fredrik Berg

Nils Rosén von Rosenstein.—Biography by Åke Dintler

Glycogenosis Type 1 (Lack of Glucose 6 Phosphatase) in Four Siblings

by G BRANTE, K. KAIJSER and P A. ÖCKERMAN

(S pplement 157)

Four siblings with glycogenosis of type 1 three of them studied for more than 10 years, are presented. In two of the patients diagnostic and other biochemical analyses were performed on liver tissue obtained by biopsy In a third sibling the type diagnosis was tentatively established by analyses on perorally sampled jejunal mucosa The clinical manifestations, which were mild, are described and the results of biochemical analyses are reported.

This is the first family with type 1 glycogenosis in which lack of jejunal glucose-6-phosphatase activity increased liver glucose-6-phosphat fructose-6-phosphate and fructose 1,6-diphosphate increased plasma glycerol and increased serum ornithine carbamoyl transferase were demonstrated

Current knowledge of type 1 glycogenosis is briefly surveyed. The writers cases are compared with other published accounts of the same disorder Theories of the mechanisms underlying the secondary biochemical disturbances are discussed. Speculations are made concerning reasons for variations between different affected families as regards severity of the clinical manifestations of type 1 glycogenosis.

ears. About two thirds of the children had ormal or only slightly bnormal EEGs at l recordings. Nearly one third showed at e first recording, low activity which rapidly minished and which must be considered be a sequel of the febrile convulsion. The w activity occurred more frequently the nger the convulsion had lasted and was observed in practically all of the children with athological neurological findings and with lergy The changes may be regarded as pending upon cerebral hypoxia as the xygen requirement is increased by pyrexia d the oxygen supply diminishes during a nvulsion particularly during the tonic ase, partly on account of respiratory arrest d partly on account of delayed circulation used by raised intrathoracic pressure Subquent convulsions occurred twice as freuently in hildren in whom the first EEG as abnormal and the incidence of subseuent paroxysmal changes in these children as ten times that in children with normal EGs. An EEG recorded during the days mediately after a febrile convulsion is us of certain prognostic value Nine of the ildren (scarcely 10%) developed transient aroxysmal changes later and these children ffered clearly from the main group in that ey showed signs of a tendency to seizures, e majority of them having a familial predisposition to convulsions and the first febrile nvulsion occurring after the age of two ears. First f brile convulsions are otherwise nusual at this age. Three out of the nine ildren have since had convulsions later uring the hitherto brief period of bservation.

DISCUSSION

C. Friderichsen When the prophylactic dministration of antiepileptic drugs to very case of f brile convulsions is under ebate, I consider that it is incorrect that e cases in which, on admission, commencing epilepsy may be suspected are not rted out first. This group comprises the hildren in whom there is (1) known familial redisposition to epilepsy or (2) focal seizures or (3) the convulsions last for over 20–30 minutes or (4) the immediat treatment has had no effect or (5) the child is over the age of 5–6 years or (6) the convulsions were not associated with pyrexia or (7) where the EEG findings were suspicious. If this group is excluded, only approximately 4% of the remaining cases of febrile convulsions will later manifest themselves as epi lepsy I am of the opinion, therefore that it is a considerable psychic stress for a mother to have to administer antiepileptic treatment for years and live in fear of possible epilepsy only because a single febrile convulsion has occurred. If the convulsion is repeated the situation is quite different and, in such cases, I would also recommend prophylactic therapy even although in our material from Sundby Hospital, after a period of observation of up to 15 years, no cases of epilepsy occurred in the 55 children who had had repeated febrile convulsions. In his time Jarlov demonstrated that in adult postepileptic alkalosis was present immediately after the convulsions. Now that methods of investigation are so easy would it not be possible to estimate the pH in the blood immediately after an attack and thus obtain a further indication of the diagnosis. — P Plum: It is excellent that a controlled therapeutic experiment such as that reported is carried out W do not consider however that children with febrile convulsions should be treated routinely as epi leptics. In the Paediatric Department Rigshospitalet, we record the EEG a few weeks after the first febrile convulsion. W only administer treatment if this EEG is abnor mal. If a child has had two or more f brile convulsions, we take prophylactic antiepileptic treatment into consideration. In such cases, we recommend prophylactic medical treatment should pyrexia occur — J Vestermdal. No difficulties are involved in getting the mothers to accept the recommendation that the children require treatment for the "febrile convulsions" provided that the condition is not termed "epilepsy — Bjørn Ibsen From an anaesthesiological point of view the significance of active and

immediate treatment of the convulsion must be emphasized. The significance of this is illustrated by a case of febrile convulsions in a child aged two years with aspiration of the gastric contents into the respiratory passages, respiratory arrest and probably cardiac arrest. The case was treated with primitive methods of resuscitation. After five hours, complet restitution was obtained. The active anaesthesiological regime includes rapid treatment of the convulsions by intravenous administration of barbiturates and possibly the employment of curare preparations, intubation and artificial respiration. It is necessary to supervise and possibly to assist the respiration in unconscious patients who have insufficient respiration without convulsions. It is equally necessary t do the same in patients in whom the convulsions involve anoxia. The EEG changes may be assessed both on the basis of the underlying causal disease and as possible anoxic sequelae which severe cerebral anoxia may produce during the convulsion, if, in addition to the actual cerebral convulsive condition, the blood supply is anoxic on account of obstruction and possible absence of adequat ventilation. The interpretation of the EEGs would probably be facilitated if the patient, as far as possible received adequat supply of oxygen. The presumption that administration of oxygen should prolong the convulsions is not in agreement with experience from electrostimulation treatment which is carried out with ventilation with pure oxygen and here the duration of the convulsions is not prolonged. Reference is mad to Knud Svendsen work on the treatment of convulsions in an anaesthetic department (*Ugeskr Laeg 124* 178, 1962) — P W BRAESTRUP: The trepidations felt in the department concerning the extra work involved by an investigation of this type proved t be fully justified and we are looking forward to its conclusion. On the other hand the preliminary experience has shown that the practical and psychological problems associated with continual administration of tablets to small children are at any rate, less than we had predicted. Some uncertainty reigns whether the children really get the tablets prescribed and, at present an attempt is being mad to illustrat this more fully The preliminary accounts have not been ble to illustrat with certainty the very important question as to whether the symptoms or investigations t the first convulsion can permit subdivision into special risk groups in view of the prognosis and the requirement of treatment and, similarly the primary question whether anticonvulsive treatment prevents subsequent epilepsy is still unsolved. The final analysis will probably illustrat these questions.

P W Braestrup: The sulphonamide concentration in th cerebro-spinal fluid after one oral dose of sulphamethoxipyridazine (Lederkyn®)

Sulphonamides are regarded as the group of bacteriostatic drugs which can pass the blood-C.S.F barrier with greatest certainty In order t illustrat this, a standard buffer dose of 100 mg Lederkyn per kg body weight was administered at varying intervals before 100 routine lumbar punctures. The sulphonamide concentration in the CSF and the whole blood was then determined in the Central Laboratory It was demonstrated graphically that the blood values varied considerably but that, by and large, these were over 10 mg% for at least 36 hours. The values showed great scatter probably on account of administration at varying times of the day and with varying relationships to meals. The average curve for the spinal concentration corresponds t this but considerably lower and values of over 6–7 mg% were only ttained in a few cases. During continuous treatment with Lederkyn, CSF values of over 5 mg% are only ttained with reasonable certainty when the total blood values are over 20 which corresponds to a 24 hour administration of approximately 50 mg per kg i.e considerably higher than the normal dosage and the dose recommended by the manufacturers. The few investigations of the sulphonamide

concentration in pathological cerebro-spinal fluids show results which do not differ in principle from the conditions found in normal CSF

DISCUSSION

J KRINGELBACH: In the Paediatric Department, The Central Hospital, Nykobing Falster since October 1958 we have employed Lederkyn as the only sulphonamide preparation in the treatment of purulent meningitis in combination with penicillin and possibly also streptomycin according to the usual rules. Our material comprises 17 cases of purulent meningitis. Thirteen of the patients were aged 5–14 months and the remaining patients were 2, 3 10 and 12 years of age Culture from the CSF revealed meningococci in 5 cases, pneumococci in 3 cases and Pfeiffer s bacilli in 3 cases. In 6 cases, no bacterial growth was obtained. Out of these 6 cases it was probable, according to the history and clinical findings, that 2 were meningococcal, one was pneumococcal, one was suspected to be a Pfeiffer infection and one suffered from B coli sepsis. All 17 cases of purulent meningitis recovered. The dosage employed was twice that recommended by the manufacturers Lederkyn being administered twice per 24 hours instead of once This double dosage was continued for a week, after which normal dosage was resumed. The results of treatment of purulent meningitis with this sulphonamide preparation are thus absolutely satisfactory

Meeting November 13 1963

O Strauch Late cases of Werdnig-Hoffmann disease

Three patients with progressive infantile muscular atrophy who had been admitted to the Paediatric Department in Sundby Hospital are reported. The patients were a of ½ years and two siblings, a girl aged years and a boy of 1½ years. In all three children the diagnosis was verified by abnormal lectromyographic findings and muscle biopsy which showed neurogenic muscular atrophy In all three children, the clinical picture was somewhat uncharacteristic as the condition was exclusively localized to the lower limbs and manifested itself in difficulty in walking and the course had been very benign. The symptoms commenced at the age of 6 months, between 6 and 12 months and at 10 months, respectively The periods of observation since the first admissions were 7 9 and 4 months, respectively and during this time no progression of the condition ha been demonstrated. In the differential diagnosis, chronic peripheral neuropathy was considered but the normal peripheral nerve conduction does not support this.

DISCUSSION

SV BRANDT: These must be cases of Werdnig Hoffmann disease but the first case at any rate, was not entirely typical. It is not unusual that the disease commences at the ages recorded.

Erna Christensen and J Vesterdal A case of Krabbe s disease

The patient a girl, was the daughter of mother aged 45 years. She had five healthy sisters and one healthy brother There were no similar cases in the family The birth and early development had been normal. Since the ge of five months, marked weakness with generalized muscular atrophy and weak tendon reflexes were observed. At the commencement of the disease, the psychic functions ppeared to be normal. The cerebro-spinal fluid was normal. Ophthalmoscopic findings were normal. The creatine phosphokinase in the serum was greatly raised *(21.2 units/litre)* The EMG suggested myogenic affection or possibly inactivity atrophy In the course of 1–2 months, periodic increase of tone occurred which was, however difficult to recognize on account of

muscular atrophy and feeding difficulties and dementia developed. The child died at the age of 10 months. At autopsy bronchopneumonia was found as the immediate cause of death.

Examination of the brain showed macroscopic sclerosis of the whit matter and patchy detachment of the cortex but no malformations. The spinal medulla had undergone flask-shaped swelling with obliteration of the normal structure and in the thoracic medulla, the central canal was dilated. Hist logical investigation revealed the typical picture of Krabbe's leukodystrophy with globoid cells in the white matter in the cerebrum, cerebellum and spinal medulla, absent myelinization and marked proliferation of astrocytes. No normal oligodendroglial cells were observed. The swollen part of the lumbar medulla proved t be a malformation with three central canals and disorganization of both the grey and white matter. The striped musculature showed diffuse atrophy but no signs of neurogenic trophy.

The pathogenesis in globoid cell leukodystrophy is mentioned. The most probable explanation is that an enzymatic defect in the oligodendroglial cells is concerned with subsequent defective myelinization and formation of the characteristic globoid cells which contain prelipoids. This thus offers a natural explanation of the fact that these patients are clinically healthy at birth and that during the first months, when myelinization should normally occur the symptoms of a progressive condition in the central nervous system develop.

DISCUSSION

Sv. Brandt: How frequently are changes found in the medulla? — Erna Christensen: I have not observed such changes previously.

O. Sønnicke. A case I diagnosis

The patient wa a girl, admitted at the age of scarcely one year to the Paediatric Department of Sundby Hospital. The clinical picture was dominated by markedly intermittent fever for over seven weeks. The general condition made cerebral lesion suspect during the first –3 weeks and, in addition, an uncharacteristic alternating exanthema and purulent nasal discharge were present for 3–4 weeks accompanied, for a period, by cervical adenitis. After hospitalization for 14 days, the child's condition was extremely poor with respiratory and feeding difficulties and parenteral fluid administration was necessary for five days. Various forms of antibiotic therapy (penicillin, sulphonamide, terramycin and chloramphenicol) were without effect. The child recovered gradually and was discharged completely well after nine weeks in hospital. On out patient follow-up examination, eight months after discharge the child was completely healthy. No bnormal results were found from the numerous investigations undertaken apart from anaemia and raised ESR. No definite diagnosis was established but the possibility of a rheumatic condition is discussed.

Villy Eriksen. Treatment of oxyuriasis with pyrvinpamoate (Vanquin®)

Forty-four children aged 11 years suffering from oxyuriasis were treated with a single dose of pyrvinpamoat (Vanquin). Eighty-six per cent were cured and there were no side-effects.

Ruth Schjødt Pedersen. A case of acetyl salicylic acid poisoning

A case of acetyl salicylic acid poisoning in a boy aged years was reported. The hild was admitted 1 hours after having consumed 9 g acetyl salicylic acid. The patient presented the typical picture of poisoning with severe disturbances in the neutrality regulation and relatively high serum salicylic acid concentration. Despite this, the clinical course was milder than in the cases previously described in Denmark. The symptoms disappeared in about 48 hours following treatment with abundant amounts f isotonic saline 5% glucosis, vitamin K and ascorbic acid.

DISCUSSION

P W BRÆSTRUP mentioned a fatal case of acetyl salicylic acid poisoning in a child aged 2 years with pneumonia. — A lively discussion (C FRIDERICHSEN P W BRÆSTRUP J KRINGELBACH, S. BRANDT O STEINICKE, B ZACHAU-CHRISTIANSEN TORBEN IVERSEN) ensued about the suitability of administration of acetyl salicylic acid to infants and small children and the participants were unanimous that acetyl salicylic acid should preferably not be administered in this age group but, if administered, the dosage should not exceed 50 mg/kg/24 hours. Further the principles of treatment of poisoning were discussed. — ERNA CHRISTENSEN It is singular that the clinical picture of acetyl salicylic acid poisoning has been recognized for 30 years but that as far as I am aware, no reports of autopsy findings in these patients are available as yet Dr Inge Tygstrup and I have each investigated a case and found toxic liver damage together with changes in the astrocytes in the white matter similar to those seen in patients with liver coma.

O Steinicke and B Zachau-Christiansen Poisoning of children with tranquillizers

On the basis of the cases of poisoning described in the literature the symptoms of poisoning which occur after ingestion of phenthiazine and chlorprotixen preparations were first mentioned Thereafter the authors own cases, a total of 6 children, were mentioned. Four of these had been admitted to the Children s Hospital, Fuglebakken after ingestion of chlorpromazine (Prozil) and two to the Paediatric Department of Sundby Hospital after poisoning with chlorprotixen (Truxal) The children, four girls and two boys, were aged between 11 months and 4 years. Serious symptoms were present in only two of the children in the form of loss of consciousness and convulsions. One of these children died 18 hours after ingestion of an apparently slight quantity of chlorpromazine The remaining four children were remote and drowsy and one was unconscious for some hours but without convulsions. Finally the treatment was discussed and the value of emptying of the stomach was emphasized even long after the ingestion of the poison on account of the prolonged disintegration time of the tablets in the stomach. The employment of emetics is recommended. On account of the mode of action of these tranquillizers, it must be considered, further whether barbiturates are the correct anti convulsive drugs in cases of poisoning such as these

DISCUSSION

S BRANDT emphasized that the practitioner prescribing such a preparation should draw the patient attention to the fact that the drug must be kept out of reach of children. — E TRAMPRUP thought that a special mechanism of closure on the medicine bottle should be devised — P W BRÆSTRUP recommended that every medicine cupboard should contain charcoal tablets.

Torben Iversen, Copenhagen

Swedish Pediatric Society

Meeting November 29 and 30 1963

Hugo Andersson, Ingrid Hagne, Barbro Johansson and Ingemar Petersén. Electroencephalography in Subdural Effusions in Infancy

EEG was pathological before operation in all of 17 cases of subdural effusions. In ten of these marked asymmetries in delt and theta-activity were registered, in three the basic rhythm was depressed and three others showed paroxysmal activity (one later developed hypsarhythmia) and one case showed "electrical silence" Postoperatively 10 out of 17 had normal EEG These patients were healthy Four of six patients who were not cured had pathological EEG before and after operation, three had paroxysmal activities and one "electrical silence"

R. Tunell, L. G. Allgén, B. Jalling and B. Persson Risks of Intoxication with Vitamin A in Infants

Five cases of chronic vitamin A intoxication in children between 3 and 5 months of age are described. They received between 17,500 and 60,000 IU vitamin A per day in water-soluble form for one to two months and became acutely ill with anorexia, hyperirritation, tender edema over the occiput and a bulging fontanelle (a sign of increased intracranial pressure). Three patients had lamellous desquamation on palms and feet X-ray of hands and feet revealed broadened and deformed metaphyseal ends and decalcified cranium. Seven-thirty days after the last dose the serum concentration of vitamin A was between 400 and 700 IU/ml (normal values do not exceed 200 IU at these ages). Follow-up examination of three cases 2–4½ years later revealed no pathological changes. Healthy children were given 7500 IU or 2500 IU of water soluble vitamin A from birth to three to five months of age. Those who received the lower dosage had a serum fasting value of serum-vitamin A of 0 ± 54 IU/100 ml which was statistically lower than the values in those who received the higher dosage (mean value 95 ± 43 IU/100 ml) Three children on the higher dose had fasting values between 185 and 200 IU but none had any sign of vitamin A intoxication Five healthy children received 22,500 IU vitamin A in a single dose; four hours later the serum level was between 350 and 750 IU/100 ml.

The results indicate that a daily dose of water soluble vitamin A should be 2500–3000 IU which corresponds to current practice in many other countries. The Swedish Medical Board will recommend this dosage for routine administration to healthy children.

DISCUSSION OF INTOXICATION IN CHILDREN

H Fegeling It is questionable if all patients poisoned with petroleum products should be intubated at gastric lavage. Before intubation a general anesthesia should be given which carries a risk of vomiting Emetic drugs may provoke vomiting when the patient is somnolent or unconscious and thus bear a risk of aspiration of gastric content.

B Hagberg A. Hamfelt and O Hansson Case with B Dependency Syndrome and Impaired Tryptophane-Metabolism

Girl born in 1959 has had infantile spasms since 8 months of age. At 15 months EEG showed hypsarhythmia. Therapy with ACTH and anticonvulsants was of no benefit. The girl was slightly tactile and deteriorated mentally At age of 3 years, therapy with vitamin B_6 was started and followed by rapid psychomotor improvement during the following year The serum content of pyridoxalphosphat and urinary excretion of

xanthurenic acid after tryptophane loading during and without vitamin B therapy were studied.

A E Roch Nordlund, J Bergst öm and E. Hultman: Muscle Glycogen in Juvenile Diabetes before and during Treatment with Insulin

Preceding reports on the contents of muscle glycogen in living man have shown great variety According to the results published by Sherlock *et al.* (1949) there is, however no obvious difference in muscle glycogen between normal individuals and diabetic patients. Using a new technique of needle biopsy introduced by J Bergström, the present authors have estimated the muscle glycogen contents of 78 normal individuals. Muscle biopsies were taken from m. quadriceps femoris. In normal individuals the mean value of muscle glycogen was found to be 1.35 g per 100 g wet muscle The normal range of this investigation was 1 0 g to 2.0 g per 100 g wet muscle weight Muscle glycogen content was determined in 10 patients aged 3 to 15 years with juvenile diabetes. Eight of them had had no previous treatment and 2 had been on insulin for 6 months. Muscle biopsies were taken and immediately followed by an injection of fast ting insulin The mean value of the patients muscular glycogen was 0.50 g per 100 g wet muscle before treatment, ranging between 0.35 g and 0 90 g per 100 g wet muscle In seven cases, control biopsies were mad 1 to 3 days fter insulin administration All these cases showed a significant increase in muscle glycogen, in five cases to normal and in two cases almost within normal limits. In nine of the patient muscle biopsy wa repeated when their diabetes was under control. In ll of these cases the values for muscle glycogen were within normal range

P A Öckerman Glycogen Storage Disease in Sweden. Critical Evaluat on of th Diagnosis

Of 36 Swedish cases, diagnosed as glycogen storage disease 21 were investigated with biochemical and enzymatic diagnostic methods. Only 5 cases were shown to ha e glycogen storage disease. In 13 cases all known types of glycogenosis could be ex cluded. Three cases could not be analyzed in det il. The results of the biochemical and enzymatic analyses are correlated to the clinical symptoms and signs and to the histopathological ppearance Clinical and histopathological investigation tends to result in more cases of glycogen storage disease than can be verified by biochemical and enzymatic methods. Some cases that had been called glycogen storage disease in spit of atypical clinical traits, might have hi therto unknown hereditary disorders.

F Karlström Gastric Ulcer in Children

A total of 184 children under 15 years of age with peptic ulcer received treatment at pediatric medical departments in Sweden during the years 1953–1962 In most cases (168) the diagnosis was confirmed by radiographic demonstration of a crater The relation between children with peptic ulcer and ther patient treated at the pediatric departments was 4 4 10,000 There has been no increase or decrease in the number of children with peptic ulcer The disease appears to be commoner in the cities than in rural districts, but is equally distributed in different parts of Sweden. In general, the incidence increases with increasing age The disease was commoner in boys (66%) than girls (34%). Duodenal ulcer accounted for 85% of the cases, gastric ulcer for 14%, and combined duodenal and gastric ulcer for 1%. Ulceration in Meckel diverticulum showed many of the same features as ulceration in the stomach and duodenum. A positive family history was present in 47% of the cases Most of these children were of normal height but thin, suggesting a leptosomatic habitus. The commonest symptoms were hunger pains, sour eruct tions or pyrosis, and vomiting attacks. Bloody om t was recorded in 31 and blood-streaked stools in 5 cases. Medical treatment was used in 169 children and surgical treatment in 15.

Two children died during the neonatal period. F llow-up investigation of 64 cases after 5–10 years observation showed that 71% of those who had been medically trea ted were either symptom-free or improved, and 19% were worse. Of those treated surgi cally 6 had been under observation for 5–10 years, and 5 of them were symptom free or improved while 1 considered that no improvement had occurred. Taking into a- count the surgically treated cases in which the observation period was less than 5 years, 11 patients reported freedom from symptoms or improvement and 1 no change in his condition. Although the series does not justify any definit conclusions from this comparison between medically and surgi cally treated patients, the figures would appear t indicat that operative treatment might be used more extensively than has hitherto been the case.

O Celander: Indirect Blood Gas Analysis through Gastrotonometry

Oxygen, carbon dioxide and nitrogen de posited in the gastric ventricle equilibrat with the blood gases. These can thus be studied by continuous analysing inflated ga mixtures. Some results in normal children and in states of hypoxia and hyperoxia were reported.

Lennart Angervall Orvar Eeg-Olofsson and Johan Stire-Söderbergh. Skin Biopsy in Dia betic Children

Skin biopsy was performed in 28 diabetic children without clinical signs of retinopathy nephropathy or neuropathy as well as in 18 controls f similar age. The diabetics also underwent EEG and EMG examinations and their ulnar and peroneal nerve conduc tion velocity was estimated The biopsy specimens were punched out from the skin on the dorsal aspect of the foot After fixa tion in Bouin solution, embedment sec tioning and staining (van Gieson-hematoxy lin, elastin, MacManus PAS) the specimens were blind coded and examined by two of the authors. A grading system was used for assessing among other things the thicknesses of capillary walls and sweat-gland basement membranes. In addition the thickness of sweat-gland basement membranes was mea sured with vernier calipers on photomicrographs with a total enlargement of about 1500 times. Whereas there were no differ ences in the thicknesses of capillary walls and sweat-gland membranes between dia betics and controls under *10 years of* ge diabetics over 10 exhibited somewhat thicker sweat-gland membranes than controls. Moreover 7 of the latter group as compared to one control had thickened capillaries. Of 5 chil dren with the thickest sweat-gland basement membranes, onset of diabetes was near puberty Average nerve conduction elocity was lower in diabetics than in controls, with slow conduction more common in diabetic children with thickened sweat-gland mem branes. Some diabetics exhibited signs of slight damage to peripheral neurones. The results indicat that diabetic microangiopathy and thickening f the basement mem branes of the sweat glands can occur in chil dren over 10 with short duration of diabetes. This could mean that puberty is of pathogenetic significance

C. G. Bergstrand, J Gentz and B Lindblad Pediatri Viewpoints on Kidney Biopsy

The results of 73 percutaneous kidney biopsies on 64 children and adolescent during the period 1957–63 were analysed. The indications and complications of this tech nique were determined. In the great majority of punctures the chief indication was to determine the diagnosis, and in a smaller number to evaluate the prognosis. The patients were divided into two groups: those with acut and chronic glomerulonephritis, and those with hematuria and proteinuria of unknown origin. Histological examination of the biopsy confirmed the diagnosis in the great majority of the former but failed t explain the symptom-picture in the latter group.

Meeting January 17 1964

J Pejme and I Silverstolpe Trial Therapy with Immuneglobulin in Cases of Infectious Mononucleosis

Treatment was carried out with gamma globulin from patient convalescing from this disease with positive Paul Bunnell and Davidsohn s absorption tests. The convalescent blood was obtained between 7–30 days after subsidence of the fever The fractionated gammaglobulin was dispensed in a 12 % solution in 0.3 molar glycocol in distilled water with pH 7 0 and merthiolate 1/10,000 employed as a preservative The immuneglobulin was administered as a deep intramuscular injection in single doses of 8 ml, corresponding to 700 ml whole blood, regardless of the patient s age and body weight. The material consists of 56 cases, with an equal number of controls, and is made up of 42 seropositive and 14 seronegative cases in the group under treatment and 43 seropositive and 13 seronegative cases in the control group. All patients received penicillin as a prophylactic against secondary infections. The sedimentation rate temperature serology and the appearance of atypical cells in the peripheral blood were observed. No difference was detected between the treated and the control groups. It seems possible that a larger dose of immuneglobulin might have produced a different result

Birgit Sköldenberg Serous Meningoencephalitis in Cases of Infection with Positive Cold Agglutination Tests

In Stockholm City Hospital for Infectious Diseases during the period January 1962–June 1963 there were 126 patients (69 women, 57 men) with cold agglutination positive infection. Of these eight (two women, six men) had serous meningoencephalitis or meningitis. Six of the eight cases were in the 5–29-year age-group (totalling 75 of the patients), and belonged to the 72 who became ill during the period October 1962–February 1963. Obvious encephalitic symptoms were present in three of the eight patients (a 14-year-old girl and 7 and 8-year old boys) The boys had severe giddiness and vomiting with changes in position. These three patients had only mild upper respiratory symptoms. Besides pneumonia, two cases also had a febrile mucocutaneous syndrome Two cases had right-side upper lobar pneumonia; pharyngitis and tracheitis occurred in one case. Five of the patients had more than a quadruple increase in cold agglutination, while the three remaining patients had titers of 16–16 256–256 and 128-neg neg. Enterovirus could not be demonstrated. One case (with cold agglutination 128, neg -neg) had neutralising antibodies against ECHO 3 without an increase in titer The patient s wife also had serous meningitis: she had ECHO 3 in feces and neutralising antibodies against ECHO 3 with increase in titer Parotitis was excluded clinically epidemiologically and in five of the eight cases, also serologically Infection from tick-born virus was excluded, as it was not that season. The time connection between cold agglutination positive infection and serous meningoencephalitis or meningitis in the eight cases constitutes no conclusive evidence of an etiological connection. As a result of future attempts to isolate mycoplasms in blood and encerebrospinal fluid, as well as at post-mortem, it may be possible to establish the etiological connection.

J Ström Wissler Syndrome

Will be published in *Acta Pædiat (Stockh)* 53 1964

E. Bengtsson and H Nordenstam. Eosinophilic Leukemia

Eosinophilic reactions occur briefly in cases of myeloid leukemia. Genuine cases of eosinophilic leukemia are however rare but they have been reported in Scandinavia by Thomsen Nordlander as well as Engfeldt & Zetterström. The latter prefer the description "disseminated eosinophilic collagen disease" to eosinophilic leukemia. There is

some obscurity regarding the classification of the disease. The differential diagnosis appears to be mainly with leukemoid eosinophilia.

Case report A 5-year-old boy presented with nasal pharyngitis and regional cervical adenitis, and after two weeks developed a picture suggestive of mononucleosis with generalized lymphadenopathy and hepatosplenomegaly Blood count showed leucocytosis with typical lymphocytes dominating After one week the eosinophils increased from 0.3 % t more than 40 %. Lymph node biopsy showed fibrous tissue with a large number of lymphatic, but also a fair number of eosinophilic cells. Sternal puncture revealed primarily eosinophils. It was of particular interest that the complement fixation test for toxoplasmosis in the 2nd, 4th, and 8th weeks showed values of 1/20 1/120 and 1/480, the dye-test was 1/250, 1/1250 and 1/6250 respectively The antipneumolysin titer: 1600, 1600 and 17 600 respectively The patient died after 8 weeks of rapidly progressing illness. Post-mortem examination revealed immature myeloid cells, mainly eosinophilic leucocytes of varying degrees of immaturity infiltrating different organs, such as the bone marrow thymus, lymph glands, spleen, liver kidneys lungs and myocardium.

L. O Kargsten Infectious Mononucleosis with Long-standing Acquired Hemolytic Anemia

A survey of literature shows that 24 of 37 cases of infectious mononucleosis with acquired, hemolytic anemia, recovered spontaneously within few weeks. The remaining three cases were splenectomized within three months after onset of disease

A case of infectious mononucleosis with auto-immune, acquired, hemolytic anemia of eight months duration is reported. A positive direct Coombs reaction occurred, which had previously been noted in 16 of 21 patients. The prolonged course of the acquired, hemolytic anemia of this case is unique, suggesting a possible underlying congenital defect of the erythrocytes, in spite of normal *hemoglobin-electrophoresis, activity of glucose-6-phosphatedehydrogenase* etc. No previous case of persistent acquired, hemolytic anemia has been described.

Meeting February 14 1964

J Houstek, Prague (guest-lecturer) School Health Activities in Czechoslovakia

B Vahlquist Enforcement f the Central Organization for Health Supervision f Children and Adolescents

Meeting March 13 1964

Panel Discussion of Renal Tubular Defects

- R. Zetterström General Aspects on Pathological Conditions of the Renal Tubuli.
- L. Hambraeus Aminoaciduria.
- J Gentz. Gluco-aminoaciduria.
- J Winberg Disturbances of the Acid Base Regulation and of the Water Reabsorption.
- R. Zetterström: Neurogenous Diabetes Insipidus.

Meeting April 4 1964

Interstitial Plasma Cell Pneumonia

- P Karlberg H. Belfrage and O Eeg-Olofsson Clinical Aspects.
- G. Ahrén Pathology
- E. Lycke and O Strannegård: Microbiology

your own country as well as abroad by virtue of your personal qualities.

The Swedish Pediatric Society is honoured to confer on you the Rosén von Rosenstein medal.

Professor Arvo Ylppö

You are legendary among pediatricians. In 1914 you carried out your first important scientific studies in Berlin and during half a century a period that at times has meant much of suffering and struggle for your country you have been a brilliant leader in pediatrics. The famous clinic in Helsinki where your distinguished pupils are now working is fine evidence of this. The clinic and the tradition you have founded have proved that even a small country far from the great thoroughfares may reach fame in the scientific world through outstanding contributions by individual workers.

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Many years ago you were a student at Uppsala, and here you took the degree of Doctor of Medicine. During the nearly 5 decades that have passed since then you have been a pioneer in both clinical paediatrics and its related fields such as social medicine. And your name is as renowned in foreign lands as in our own. You have founded a school, and today all the professors of pediatrics at the Swedish universities are directly or indirectly pupils of yours. All of us respect you warmly for your personal qualities, and we admire you for your contributions to our specialty. No other Swedish doctor since Rosén von Rosenstein a day has so brilliantly as you represented pediatrics.

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Finally I would on this occasion quote a few words from Arvid Wallgren biography of Rosén von Rosenstein in our memorial book:

Without doubt Nils Rosén von Rosenstein occupies a very prominent place among the great men of Sweden. The lustre he spread over our country still glows and we pediatricians owe him great gratitude. We endorse with all our heart the hope that was expressed by the American pediatrician and medical historian John Ruhräh in an oration in the Cathedral of Uppsala in 1930 to the memory of Nils Rosén, 'that the spirit that animated the Father of Pediatrics in Sweden should forever remain among its modern practitioners around the world'

The Swedish Pediatric Society has also conferred the Rosén medal on Professor Robert Debré, then on a mission to Canada. Professor Arvid Wallgren at a meeting of the Executive Board of the International Children's Center in Paris June 11 1964, on behalf of the Society delivered the medal and the jubilee volume with the following words:

"Il y a deux semaines l'Université d'Upsal et la Société de Pédiatrie Suédoise ont célébré le deux centième anniversaire de la publication du 'Traité de Pédiatrie' du Professeur Nils Rosén von Rosenstein. Ce livre a joué un rôle éminent dans le développement de la pédiatrie non seulement de notre pays mais de toute l'Europe contemporaine. Il été publié en 8 langues et a paru dans une vingtaine d'éditions. A l'occasion de cet anniversaire, l'Université et la Société de Pédiatrie ont publié un livre de jubilé et la Société de Pédiatrie a inauguré une médaille de Rosén. La Société m' chargé de transmettre ce livre de jubilé et la médaille de Rosén à vous, Monsieur Debré, et de vous présenter sa profonde admiration et son témoignage au Maître de la Pédiatrie européenne. Nulle personne vivant n'a fait autant que vous pour l'amélioration de la santé des enfants et pour le développement de la pédiatrie mondiale par vos travaux scientifiques et votre activité comme Président du Centre International de l'Enfance

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A. Wallgren (Stockholm) Rosén on Rosenstein Biography

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BOOK REVIEWS

A W Wilkinson (ed): Recent Advances in Paediatric Surgery

Churchill Ltd., London, 1963 300 pp 50S.

"This book should help postgraduates working in the field of pediatrics, general surgeons dealing with children, and the specialist pediatric surgeon, to evaluate recent developments on a broader front than is possible from the most detailed review of current practice in a single hospital

Ten contributors, 8 of which from The Hospital for Sick Children at Great Ormond Street London, cover a great deal of debated problems in pediatric surgery Prof A. W Wilkinson, the editor is through his many publications on metabolic problems including fluid-electrolyte disturbances the leading name in that field. Other contributors are also well known experts. Even if the advances are not exactly recent the book is recommended. As a progress in clinical publication of this type is noted chapter on clinical genetics, a field of increasing interest

A O Ericsson Stockholm

W Tönnis and G Friedman Das Röntgenbild des Schädels bei intrakranieller Drucksteigerung im Wachstumsalter

107 pp. Springer Verlag 1964. Berlin–Göttingen–Heidelberg Price DM 58—

This book is the result of a roentgenologic study of the signs and symptoms of intracranial hypertension in 626 patients less than 20 years of age Its primary aim is t determine the value of conventional roentgenography of the skull in the detection of the presence of intracranial hypertension and evaluation of its cause About three-fourths of the patients had intracranial tumors. In the remainder the etiology was diverse and included non-tumorous stenosis of the aqueduct and foramen of Magendie tuberculous meningitis, abscess f the brain, encephalitis and craniostenosis.

In the introductory chapter the roentgen findings in normal skulls of growing individuals are described and the measurement criteria are defined. This section is followed by an account of the roentgen manifestations of intracranial hypertension, detailing their individual relationships to the location,

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type and time of appearance of the pertinent intracranial disorders. Emphasis is given to those findings which enable an early diagnosis to be established. The final section deals with the postoperative roentgen appearance of the skull. The illustrations are invariably instructive and deserve high praise for their quality. The list of references is extensive and up to date. In the reviewer's opinion this book is an important contribution and is warmly recommended.

Ulf Rudhe, Stockholm

H. L. Vis: Aspects et Mécanismes des Hyperaminoacidúries de l'Enfance. Recherches sur le Kwashiorkor le Rachitisme commun et le Scorbut. Preface by R. Dubois. Summary in English.

Éditions Arscia S.A., Brussels, 1963, 325 pp. Price Fr B 540.

According to the author: "The main purpose of the work was to define the free amino acids in urine, plasma and striated muscle of children and to investigate the pathogenesis of the hyperaminoacidurias in three deficiency diseases: kwashiorkor, rickets and scurvy." Standard methods were used, free amino acids being determined by the chromatographic methods of Moore & Stein, 1954, and of Spackman, Moore & Stein, 1958.

The absolute amount of urinary free amino nitrogen increased regularly until puberty. The amino acid pattern varied somewhat according to age. In children between 9 months and 2 years of age (20 cases) glycine, histidine, glutamine, alanine, serine, lysine, 1-methylhistidine, taurine, threonine and β-aminoisobutyric acid amounted to 87% of the total amino acids, the other amino acids were present only in small amounts. In children between 4 and 6 years of age (7 cases) the excretion of 1-methylhistidine, taurine, cystine and glycine increased more than the excretion of other amino acids, a change which was still more obvious in children between 8 and 10 years (5 cases). The amino acid pattern in the urine of those older children resembled that observed in adults.

The concentration of amino acids in plasma of children between 9 months and 2 years of age (20 cases) was generally lower than reported for adults, especially the concentration of cystine, glutamic acid and glutamine.

The pattern of free amino acids of striated muscle was different in children (4 cases) and adults (2 cases). The most striking difference was a higher level of taurine and a lower level of glutamine in the children.

The hyperaminoaciduria was very marked in rickets, only moderate in scurvy and latent in kwashiorkor where it appeared only when alimentary protein was given. In all three conditions there was an almost uniform increase in the excretion of the amino acids constituting 87% of the physiological aminoaciduria. However there was a proportionally low excretion of taurine in rickets, and a notably high excretion of taurine and β-aminoisobutyric acid in kwashiorkor and of β-aminoisobutyric acid, proline and hydroxyproline in scurvy. The large quantities of taurine in urine, plasma and muscle of the children suffering from kwashiorkor was thought to be due to an accelerated conversion of the sulfur amino acids into taurine or to a slowing of the desamination of the latter. The presence of free proline and hydroxyproline in scurvy indicated an alteration in the metabolism of connective tissues. The excess excretion of β-aminoisobutyric acid depended on an increased catabolism of DNA and on a slowing of cellular mitosis.

In all the deficiency conditions studied the aminoacidurias were thought to depend mainly on disturbances in the intracellular osmotic pressure and to cellular intolerance of a few amino acids. On the basis of these conclusions the author proposes a new classification of aminoacidurias into four types: Hyperaminoacidurias due to electroosmotic disturbances (1), tubulopathy (2), renal overflow (3) and congenital metabolic blocks (4). Conditions belonging to the fourth type are summarized in a surveyable table.

The book which contains a comprehensive and thorough review (291 references) of the literature is wholeheartedly recommended as a most useful source of information for those who are interested in this field.

Rudolf Jagenburg, Gothenburg

Acta Pædiatrica 53: 501–504. Nov 1964

From the Department of Child Health, The University of Sheffield, England

Salicylate Poisoning: The Diagnosis when Its Possibility is Denied by the Parents

by DOUGLAS PICKERING

When a child is found to be in coma, a variety of causes have to be considered. One of these is poisoning. The diagnosis of poisoning is apt to be discarded if the possibility is persistently denied by the parents, and the results of the mistake are likely to be disastrous. The following three cases illustrate the point.

Case 1

O. U., aged 1½ years, was admitted t a peripheral hospital with a history of cough for 24 hours, and rapid deep respirations for 3 hours. There was no history of poisoning. On physical examination, she was pale and mildly dehydrated. Her temperature was 36°C. Her pulse was 126 per minute. Her respiratory rate was 60/min. The breath smelled of acetone. No other abnormality was noticed. The urine revealed a trace of reducing substances. Rothera test showed large quantity f acetone. Her electrolytes were—sodium 149 mEq/l, potassium 3.4 mEq/l, chloride 121 mEq/l, bicarbonat 9.7 mEq/l, blood sugar 334 mg%. On the basis of these findings diagnosis of diabetic ketosis was made. This was treated with intravenous 1/6 sodium lact t t 100 ml/hour. She was given 1 mega unit of crystalline penicillin, a gastric tube was placed in position and aspirated hourly. She was given soluble insulin 10 unit subcutaneously and 10 units intravenously. Four hours later her respirations were improving. She was awake and taking notice of her surroundings. Her urine, however showed no change from the above values, and so the dose of insulin was repeated. One hour later however she had become comatose flushed and sweating. She was thought to be hypoglycaemic, and 25 ml of 50 % glucose were given intravenously. There was a slight improvement in her condition. Her electrolytes were repeated, and showed sodium 155 mEq/l, potassium 2.. mEq/l, chloride 112 mEq/l, bicarbonate 22 mEq/l, blood sugar 110 mg %. Her urine showed no reducing substances and there was only trace of acetone. Over the following hour she progressively deteriorated and died despit oxygen and 1 ml of intramuscular Nikethamide.

The following day under direct questioning, the mother recalled that on the evening prior to admission, she had found the child playing with a bottle containing aspirin tablets. The bottle (which was not in fact an aspirin bottle) was usually kept in the top drawer f the sideboard, and the children would have seen it put there on previous occasions. She said she was not certain whether the child had swallowed any tablets, but she gave the child salt water in an unsuccessful attempt t induce vomiting. Salicylate levels were estimated on sample of blood taken on admission, and the blood salicylate level was found to be 51 mg%. Post mortem findings confirmed the cause of death as cerebral oedema due t salicylat poisoning.

Case 2

R. O., aged 6 years, was admitted with a history of cough for 48 hours, and rapid respirations for 2 hours. Half-an hour prior to admission, she had awakened looking very ill, and had become stiff" and did not respond to her mother calls. She was seen by her family doctor who noticed her limbs to be twitching slightly and referred her to hospital. On admission she was stuporose, with slight jerking movements of both arms. Her temperature was 39°C There was a tachycardia of 160 per/min. The blood pressure was 100/48. The respirations were rapid and deep Her complexion was pale her lips were cyanosed, and the pupils tightly contracted. After they had been dilated with atropine the eye grounds were found t be normal. The tongue was pale and dry and there was tenacious brown mucus over the fauces. Mild dehydration was present. Examination of the central nervous system showed generalised hypotonia and bsent reflexes. On auscultation of the chest, some fine rales were heard over the left base. The patient was catheterised; the urine was clear there were no reducing substances, but acetone was present in large amounts. The urine was tested for salicylates, despite the history that not more than two t blets f junior asprin had been ingested. The salicy lat test was strongly positive. While this investigation was being carried out the patient became comatose. She was treated as a case of aspirin intoxication, and was given M/6 lact t intravenously She improved initially Her breathing became less acidotic in type. She vomited altered blood twice. After receiving 180 ml of fluid intravenously she had a generalised convulsion and died. She had received the following drugs in hospital. on dmission paraldehyde 2 ml, penicillin ½ million units intramuscularly and hydrocortisone 25 mg intramuscularly The cerebrospinal fluid was normal apart from a slightly raised protein of 40 mg% A specimen of blood taken when she was admitted revealed sodium 152 mEq/l, potassium 7 mEq/l, blood urea 75 mg%, carbon d xide 13.9 volumes%, serum salicylate 52 mg%. Post-mortem analysis of the tissues showed that the body contained between 2 and 3 g of aspirin. The parents continued to maintain that it was impossible that the child could have obtained more than 2 junior aspirin tablets. (A junior aspirin contains ¼ mg of aspirin.)

Case 3

L. E. M., aged 2 years, was admitted to a peripheral hospital, with a history of being off colour for 2 days, vomiting for 12 hours, and having a progressive increase in the depth of respirations for 3 hours. Physical examination revealed no abnormality Chest x ray was normal. Her serum electrolytes were sodium 122 mEq/l potassium 4.6 mEq/l, bicarbonat 5 mEq/l, chloride 103 mEq/l, blood urea 70 mg% blood sugar 300 mg%. In her urine there were no reducing substances, but large quantities of acetone. A diagnosis of diabetic ketosis was made, and she was given 20 units of soluble insulin. Her blood sugar fell t 35 mg% and she had a convulsion. A lumbar puncture was performed, which revealed a C.S.F containing no cells, protein 30 mg%, chloride 746 mg%, sugar 44 mg% An infusion of 5% dextrose saline was commenced. Twelve hours later she had a further convulsion. Her blood sugar had fallen to 40 mg%. Her electrolytes were sodium 122 mEq/l, potassium 4 mEq/l, bicarbonate 11 mEq/l, chloride 102 mEq blood urea 48 mg%, serum calcium 6.5 mg%. She was given calcium gluconate intravenously as the convulsion was thought to have been due to hypocalcaemia. At this point advice was sought from the Department of Child Health, t the Children Hospital Sheffield. It was suggested that she might have salicylat poisoning and that a history of this specifically should be asked. It was stated that the child might have been given 2 ad lt aspirin tablets (5 mg each) but that the girl could not have obtained any more, because the bottle was on a shelf out f reach of the child. It was suggested that she should be transferred t the Children s Hospital as a possible case of salicylate poisoning in spite f the

above story She was transferred, and the blood salicylate level was found to be 50 mg %. The child was flushed and appeared febrile. Her temperature was 38.5°C. She was well hydrated She responded to painful stimuli. Her pulse was 144 per minute Examination of the chest revealed widespread rales and rhonchi over both lungfields. Her abdomen revealed no abnormality Examination of the central nervous system showed no other abnormality She was treated with 10% dextrose saline containing 1.26% sodium bicarbonate and sol blo penicillin 500 000 units 6 hourly After 600 ml had been given she had regained full consciousness, and was able to take fluids by mouth. She was given sodium bicarbonate 0.3 g t.d.s. Twenty-four hours later she was up and about in the ward, and two days after admission she was discharged home fit and well.

Discussion

In all cases the diagnosis was rendered difficult by the parents denial of the possibility of salicylate poisoning An additional difficulty was the finding of a high blood sugar and the presence of acetone in the urine, suggesting the possibility of diabetic coma The high blood sugar may be caused by the action of salicylates in blocking certain of the later stages in glucose metabolism Fatty acid catabolism takes place and ketone bodies are formed. The urine may give a positive result with Benedict s test, either because of a true glycosuria or because of the weakly reducing action of the excreted form of the salicyl group The violet colour in the urine after adding ferric chloride due to the breakdown products of salicylates, can be confused with the pure burgundy red produced by aceto-acetic acid Rothera s test is negative unless a genuine ketosis had developed—as it may do as a result of the disturbance of carbohydrat and fat metabolism [ ]

The clinical diagnosis should be suspected when a child presents with unexplained over ventilation. The absence of sugar in the urine or the presence of only a small amount of reducing substance should alert one to the diagnosis. The diagnosis is confirmed by estimating the serum salicylate level

It is interesting to speculate as to the reasons for the parents denial of the possibility of salicylate poisoning The parents may deny it because they are genuinely ignorant of the fact that the child has ingested the poison. This may be a reflection of their lack of care in their storage of medicines. They may deny it because they do not want to recognise the true diagnosis feeling guilty about the matter They may deny the fact because they realise the possible legal implications—either in their being accused of neglect and carelessness or of actually poisoning the child.

A simple screening test for salicylate poisoning has recently been described [1]. This should be used when there is any possibility of the child s symptoms being due to salicylates.

Summary

When a child passes into coma the possibility of poisoning should be considered, even though it is denied by the parents. The presence of overventilation should suggest the possibility of salicylate poisoning The confusing finding of a high blood sugar is mentioned Three cases of salicylate poisoning are described. In all cases the possibility of poisoning was denied by the parents. The possible reasons for the parents denial were discussed.

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Disturbances of Hydrogen Ion Balance Occurring in Premature Infants

I Early Types of Acidosis

by POUL KILDEBERG

For many years it has been a universally accepted, though rather lonesome truth that the acid base parameters of newborn infants differ to the acid side as compared with those of normal adults. Without questioning the existence of frank acidosis in the neonatal period recent writers have proposed the view that healthy newborn infants are not acidotic *sensu stricto* but normally adjusted to lower levels of blood pH and bicarbonate concentration, possibly reflecting a relative acidosis of the maternal environment during gestation [24 32].

Again, when compared with term infants the acid base level of newborn prematures is generally considered to be displaced farther down the pH scale and evidence in support of this has been published by several authors [5 22 33]. However in view of the increased incidence of perinatal complications associated with premature birth and the possibility of a corresponding rise in the number of acid base derangements it might be anticipated that paradoxically more definite knowledge of the nature causes, and incidence of such disturbances might help to clarify further a concept of normo-acidity in premature infants. During the studies reported here it became possible to define several entities of acid base derangement in a group of premature infants and from the acid base point of view to add one quality to the rather ill-defined term 'healthy premature'

Case Material

Four hundred and eighty two determinations of the acid base status were made in 128 infants with birth weight (BW) < 2500 g, treated at the Obstetric and Pediatric Departments of the Odense County and City Hospital during the period May 1st 1961–October 1st, 1963. These 128 premature infants represent 31% of the total number of prematures seen at these departments during the past two and a half years. Twenty six infants had a BW < 1500 g, the mean BW for the whole case material being 1854 g

Infants weighing less than 1500 g were preferably treated in incubator Oral feedings with glucose in water were started 24–48 hours after birth, depending on the BW Fifty-four infants showed completely normal clinical perinatal course 47 surviving

prematures suffered more or less severe complications while 27 infants died before a weight of 2500 g was reached. Twenty-two infants were asphyctic at birth. In 19 typical "respiratory distress" was noted during the first days of life

Random selection of cases for study was not attempted. Likewise, blood samples were not drawn at regular intervals from all infants studied throughout the stay in hospital. In 38 prematures only one or two determinations were made at different times after birth.

Methods

Using capillary blood obtained by heel prick, determinations of the acid base status were carried out by means of the Astrup micro equipment [1 23]. Measurements of the actual pH were made in duplicate and double equilibrations were performed at both high (appr 60 mm Hg) and low (appr 30 mm Hg) CO tensions. By the "base excess (BE) is indicated, in mEq/l the titratable base or acid (negative values) of the blood (end point pH 7.40 at pCO_2 40 mm Hg)

Correction of the pCO values for subnormal oxygen saturations were not made. Under ordinary circumstances, the error involved herein is not serious, but in cyanotic and acidotic neonates it may become very considerable (pCO values too low by 10–40%) However because the present approach involves primarily a comparison of different groups of cyanotic and of different groups of non-cyanotic infants the data will still permit of certain conclusions.

Measurements of blood lactic acid concentrations were made by the method of Horn & Bruns [12] using the Boehringer test-set.

Results

Fig 1 shows the results of 243 determinations of the acid base status made in 102 premature infants aged 0–7 days. The hexagon indicates the normal range for term infants as computed from acid base measurements undertaken in 90 normal infants during the first week of life It is seen that this range fits the values obtained from healthy prematures rather closely the main difference lying in the more frequent occurrence of high pCO_2 values following premature birth, and it would appear that the metabolic acid base characteristics of less than one week old, healthy' prematures conform to those of normal term infants,—at any rate when healthy refers retrospectively to an uncomplicated course throughout the perinatal period. A mean BE of −2.60 mEq/l (σ = 0.343) and a mean pCO of 37 7 mm Hg (σ = 1 173) were computed from 68 determinations of the acid base status made in 36 healthy premature infants (mean BW 1866 g) during the first week of life (open circles of Fig 1) the corresponding values for the term infants being −1 94 mEq/l (σ = 0.238) and 36 4 mm Hg (σ = 0 489) respectively These sample means are not particularly suited for statistical comparison but the observed differences between premature and full-term neonates are at any rate not significant For the BE values the difference is not significant at the 0 1 level (t = 1.568 $t_{0.1}$ = 1 657 127 degrees of freedom) Some values falling within the normal range were obtained from infants who later died. These deaths mostly resulted from congenital diseases, i.e cardiac cerebral, and other malformations.

The figure further demonstrates that severe disturbances of hydrogen ion homeostasis occur frequently in premature neonates, the typical disturbance being one of combined—i.e respiratory and metabolic—acidosis. A combined acidosis is essentially an uncompensated disturbance

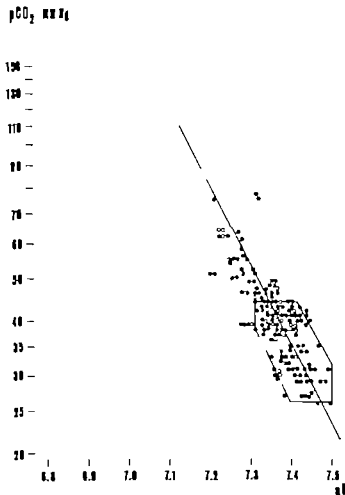


Fig. 1 Result of 243 determinations of the acid-base status undertaken in 102 premature infants during the first week of life

○ Acid-base values f infants who either had completely uncomplicated perinatal clinical course or went through minor difficulties (slight distress, single short cyanotic episodes, moderate icterus). ⊙ Acid-base values of surviving prematures who suffered more or less severe complications (intrauterine or extrauterine asphyxia, atelectases, respiratory distress, dysmaturity brain damage, severe icterus) or diseases (erythroblastosis, malformations et). ● Acid-base alues of infants who died before weight of 2500 g was reached.

The hexagon indicates the normal range ($\bar{X} \pm 2s$) for term infants as computed from values obtained from 90 normal infants aged 1 7 days: Mean base excess (BE), $\bar{X} = -1.94$ mEq/l standard deviation, $= 2.21$ standard error of the mean, $s_{\bar{x}} = 0.238$. Mean pCO_2, $\bar{X}$ 38.4 mm Hg $= 4.64$, $s_{\bar{x}} = 0.489$ Mean pH, $\bar{X}$ 7 403, 0.0469 $= 0.00494$. The normal CO_2 bsorption line corresponding to hemoglobin concentration of 70 g/100 ml is shown.

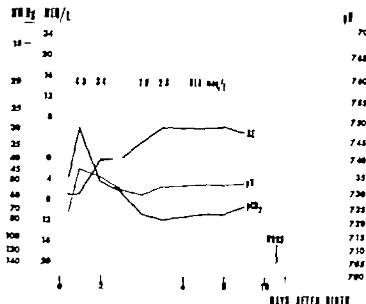


Fig. 2. Course of acid base parameters in a small male premature weighing pproximately 800 g O 1609 6. -62. Apart from mild initial asphyxia, the infant kept normal colours through the first 30 hours of lif Subsequently cyanotic attacks became frequent. Moderat edema was noted but did not increase and the diuresis appeared satisfactory On the 3rd day serum N was 156 mEq/l and serum K 5.2 mEq/l. On the 8th day urine Na was 106 mEq/l and urine K 1.8 mEq/l.
Treatment: Incubator oxygen, glucose solution, 5-10 %, intravenously human albumin, 20 %, 45 cc intravenously in the course f the first 4 days. A topsy was not performed. (BLA: Blood lactic acid.)

tending to produce very low pH levels. The lowest pH value found in a living infant was 6 68 the lowest figure obtained from a surviving infant being 6.89 It is seen that in infants with pCO_2 values less than about 70 mm Hg the metabolic com ponent is usually moderate while still higher pCO levels are accompanied by rapidly increasing metabolic acidosis. Fur thermore a fairly good correlation was found between the severity of the acid base disturbance and the clinical course During the second and following weeks of life the pattern of acid base pathology tends to change somewhat this change being largely due to a more frequent oc currence of isolated metabolic acidosis [16].

From a closer study of these observations emerged the classification of acid base disturbances associated with prema ture birth used in this and a following report [16] The incidence of the different types of disturbance in the present case material probably gives some idea of the relative frequencies, but the selection of cases for study allows no statements concerning the true prevalence of these abnormalities in premature infants

Respiratory acidosis (10 cases) was occasionally observed during the first 4 hours of life or in the early stages of respiratory difficulties, but in nearly all cases of protracted hypoxia metabolic acidosis supervened. However respiratory acidosis of several days duration was observed in

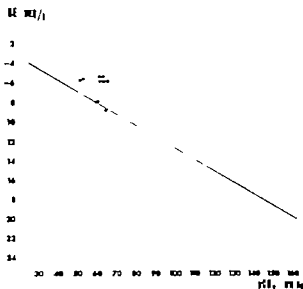


Fig 3. Regression of base excess (BE) on pCO_2. Forty-eight determinations of the acid-base status made in 20 premature infants with combined neonatal acidosis.

$$BE = -1.02 - 0.116\ pCO_2, \quad r = -0.73, \quad P < 0.001$$

two very small prematures weighing 990 and 800 g respectively (Fig 2). No explanation of this apparent difference from the response seen in older premature infants was found. It is tempting to conclude from the data of Fig 2 that in this 26–28-week-old fetus the renal response to an elevated pCO level was well developed. In one infant weighing only 750 g who died three days after birth, mild combined acidosis was present during the first 48 hours. The last sample taken showed an almost normal acid base status (Fig 5a).

The *combined* respiratory and metabolic *acidosis* (29 cases) was found to be the most characteristic and most frequently occurring disturbance of neutrality regulation. Although it may be present at any time during the first two weeks the more severe cases were largely confined to the first days of life. In asphyxiated newborn infants combined acidosis of more or less severe degrees was an almost obligatory finding and, conversely severe combined acidosis was invariably associated with cyanosis and poor respiration. A highly characteristic feature of this acidosis was the rapid fluctuations occurring in the BE as well as in the respiratory component (Fig 6b–c). As mentioned above the metabolic acidosis appeared to be in some way correlated to the development of hypercapnia. A total of 48 determinations of the acid base status were made in 20 patients with combined acidosis, and correlation analysis on these data (Fig 3) proved the BE and pCO_2 values to be strongly and significantly correlated $r = -0.73$ $t > 7$ $t_{0.001} = 3.52$

The above relationships the very nature of the combined acidosis, its variability

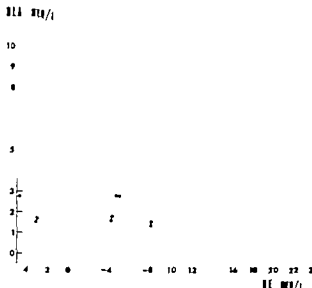
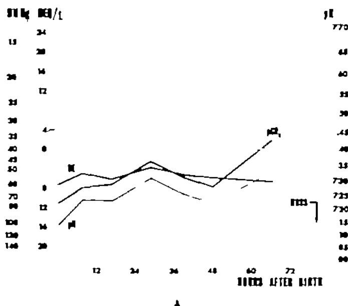


Fig 4. Fifty-nine determinations of the blood lactic acid concentration (BLA) in 30 prematures with early neonatal acidosis.

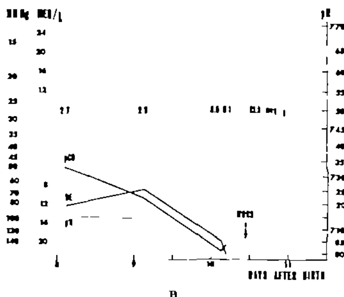
as well as its correlation to the age and clinical condition of the patients classify this particular disturbance as one measurable manifestation of neonatal asphyxia, caused, in the newborn, by severe depression of the alveolar ventilation. Following intrauterine asphyxia, both components of the acidosis may be present at birth.

Because of the association between the pCO_2 level and the degree of metabolic acidosis and earlier reports on increased blood levels and urinary losses of organic acids in newborn infants [3 5 8 9 21] determinations of the blood lactic acid (BLA) were made in a number of acidotic infants in an attempt to elucidate a possible contribution of anaerobiosis to the accumulation of non volatile acids. Fifty nine determinations of the BLA were carried out in 30 infants with neonatal acidosis of varying degrees While BLA/BE (Fig 4) and BLA/pCO_2 plots failed to show any correlation of the BLA level to mild and moderate degrees of acidosis, severe, life-threatening acidosis was constantly accompanied by a considerable increase in the BLA. Even under such circumstances, however the increase in BLA could account for not more than about one third the acid excess present (Fig 4 and 5B) Nevertheless, these results, the strong correlation between the respiratory and metabolic components of the acidosis, and the occurrence of rapid fluctuations in the BE level unquestionably favours the view that the early metabolic acidosis is a secondary phenomenon, at least in part due to the accumulation of combustible organic acids during hypoxia However an internal redistribution of H^+ between the cellular and extracellular compartments may be another possibility deserving of consideration, cf discussion p. 514

Unfortunately the lack of correction for subnormal oxygen saturations does not



A



B

Fig. 5. Cases of combined acidosis. A, Premature girl weighing 750 g, O 1462/61–62. No asphyxia or respiratory distress. Treatment: Incubator oxygen, glucose. Autopsy: Lungs well aerated; cerebrum and adrenal glands appeared normal. Histologic examination of the umbilical cord showed signs of intraovular infection. B, Premature girl weighing 1990 g J *6_/62–63. Admitted 7 days old with history of meconium-stained amniotic fluid, neonatal asphyxia, and persisting respiratory difficulties. Chest roentgenograms and postmortem examination showed severe atelectasis.

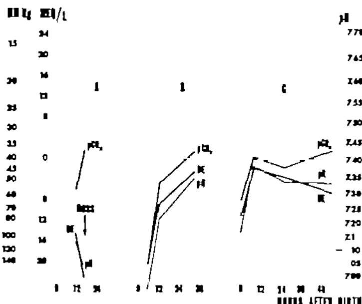


Fig. 6. Types of early acid base disturbance. A, Premature male infant, birth weight 1800 g. J *64/61–62. Cried and breathed normally at birth. Subsequently severe respiratory distress developed. B, Premature female infant, birth weight 1600 g, O 789/61–62. Asphyxia at birth (Apgar score 6 ‡ one minute). Incubator oxygen. Rapid clinical improvement. C, Premature female infant birth weight 1500 g, 0 669/61–62. Normal delivery and uncomplicated course through the first 24 hours. During the second day diarrhea and beginning metabolic acidosis supervened.

permit of exact classification of borderline pCO_2 values in cyanotic patients. However during the study of the acid base values of these premature infants it became clear that in a certain group of acidotic infants the pCO_2 values sometimes fell short of the correlation to the BE values discussed in the foregoing, i.e. the disturbance presented as a *predominantly metabolic acidosis* (Fig 6A and 7). It is seen in Fig 7 that following a brief initial phase of combined acidosis the BE remains low through 24 hours in spite of an apparently normal pCO_2 level, a situation different from those depicted in Fig 5A–B and 6B–C. Metabolic acidosis of this type was observed in 10 premature infants in 8 of whom a diagnosis of "respiratory distress" could be made. Of the remaining two patients one had only slight acidosis, and one died two days old from severe cardiac malformation. In such distressed and acidotic infants heavy cyanosis of the blood in the presence of an apparently normal pCO_2 was observed frequently.

Several mechanisms require consideration when attempts are made to account for the development of predominantly metabolic acidosis in cyanotic patients. Large veno-arterial shunts, uneven distribution of pulmonary ventilation/perfusion ratios [6] as well as diminished pulmonary diffusion capacity ("alveolo-capillary block syndrome" of Austrian *et al* [ ]) might contribute to the development of hypoxia *without significant CO_2 retention*. Whereas studies in the respiratory physiology of prematures with respiratory distress [15

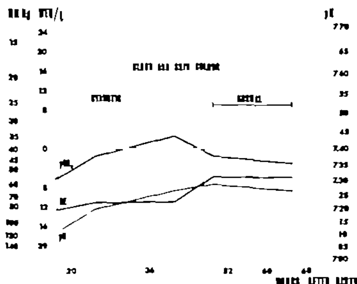


Fig. 7 Predominantly metabolic acidosis in premature male infant, birth weight 1840 g, 0 736/61–42. Delivered by Caesarean section (placent praevia), cried and breathed normally within one minute. Typical respiratory distress developed. Treatment: Incubator oxygen, Alevaire[1] glucose

25, 31] seem to bear out the significance of RL-shunts and ventilation/perfusion inequalities, evidence concerning the resistance to diffusion in respiratory distress is scant and indirect From a histopathologic point of view an "alveolo-capillary block might be expected to equivalate physiologically the increased alveolo-capillary distance

In the case of low or decreasing pCO_2 values in the presence of profound acidosis and hypoxia (Fig 6A), a fourth possibility may be considered. A normal and steady pCO_2 reflects a perfect balance between net CO production and pulmonary CO excretion, and pronounced anaerobiosis might be expected to disturb this equilibrium by causing a temporary decrease in the total cellular output of CO_2, opposing the hypercapnia or e en causing the pCO_2 to fall.

Finally it is to be emphasized that several infants with a typical respiratory distress syndrome suffered severe combined acidosis especially during the early phases of th illness which probably reflects the contribution of atelectasis and hypoventilation to the oxygen debt

A *late metabol c acidosis* occurring in apparently healthy prematures during the second and following weeks of life as well as acid-base disturbances related to complicating diseases will be the subject of another report [16]

Discussion

The results obtained have been considered in some detail in the preceding section only a few points shall need further comment It is evident from the data reported here that presence or absence of a special anaerobic metabolic set-up is not required to explain the small if not insig

nificant difference between the metabolic acid base status of healthy premature and term infants. Any such difference appears to be one of degree rather than of kind. It is by now well established [4 14, present investigation] that asphyxia of some duration will lead to metabolic acidosis, whether or not due to anaerobic glycolysis, in prematures as well as in term infants. Accordingly the greater incidence of complicated deliveries and asphyctic insults associated with premature birth will necessarily influence rude averages of BE in such a way that newborn premature infants turn out to be acidotic as compared with term infants. Likewise if a comparison is made in 2–3 week-old infants the late metabolic acidosis [10] may be expected to disturb the results. In the present investigation, segregation of the cases under study according to simple clinical criteria brought the mean BE of 'healthy premature and term infants very close together—in fact within the limits of statistical significance

Considering the metabolic acidosis of *asphyctic* neonates, it is to be emphasized that a main feature of this disturbance is its complete reversibility upon proper oxygenation of the tissues. Accumulation of combustible organic acids has generally been held responsible for the relative acidosis of newborn infants [5 21 33 35] but surprisingly little evidence is available concerning infants in actually asphyctic states. Eastman & McLane [7] showed excess lactic acid in the blood of newborn asphyctic infants to be of fetal origin, but a quantitative comparison of the "anaerobiosis as estimated from the amount of organic acids present in blood and urine and the metabolic acid base status of such infants has not been published. Like those of Vedra & Ulrych [20] the results of the BLA determinations undertaken in the present study do not lend much support to a concept of "anaerobic acidosis as the sole cause of the low BF values regularly found in asphyctic premature infants They rather suggest accumulation of LA to be a terminal phenomenon Obviously acids other than lactic may be involved too, but the lack of correlation between the BE and BLA in infants whose acidosis is not very severe seems to disfavour this possibility as the full explanation. In an attempt to explain the metabolic acidosis observed in asphyctic adults [11] Weisbrot *et al* [32] mention the possibility of a shift of H^+ from cells to plasma due to hypercapnia and suggest that such a mechanism might also apply to the acidosis of newborn infants. The demonstration of predominantly metabolic acidosis in prematures with respiratory distress in the present study would seem to speak against an elevated pCO_2 as the cause of such internal redistribution of hydrogen ions As shown in experiments by Siggaard Andersen [23] and others, *in vivo* CO_2 absorption lines differ a little from the corresponding *in vitro* ones, but the observed difference can only account for slight reduction of the BE during *in vivo* hypercapnia In 1922 Warburg [30] showed intra and extracellular H of the blood to be distributed according to the red cell membrane potential. Depolarization of the cell surfaces during profound hypoxia might result in cellular hydrogen ions leaking into the plasma and it would appear that hypoxia rather than hypercapnia is a likely cause of such shift

The reversibility of the neonatal meta

bolic acidosis raises therapeutical problems. Obviously "titration" of acidotic premature infants with sodium bicarbonate as recommended by Hutchison *et al* [13] and by Usher [26 27] involves grave risks of overcorrection and alkalosis, which offers a complete analogue to the overcorrection of diabetic acidosis discussed by Møller [19]. In infants with reduced alveolar ventilation, i.e. limited capacity for pulmonary CO_2 excretion, very large doses of bicarbonate are required to neutralize the blood or extracellular compartment and overloading with sodium and fluid as well as aggravation of the impending hypercapnia constitute further drawbacks of which anyone intending to treat prematures with concentrated bicarbonate solutions should be aware.

Conclusions

1 During the first postnatal week, the metabolic acid base status of premature infants having a completely normal perinatal clinical course appears not to differ significantly from that of normal term infants

2 In premature newborns (as in term infants) asphyxia of some duration leads to reversible metabolic acidosis In asphyxia due to hypoventilation a combined respiratory and metabolic acidosis results. In such cases the base excess and pCO_2 values are strongly correlated.

3 In some infants with "respiratory distress" the disturbance takes the form of a predominantly metabolic acidosis.

4 The metabolic acidosis appears to be a direct consequence of the hypoxia. Accumulation of combustible organic acids (chiefly lactic) contributes to the acidosis in the terminal phases of asphyxia but other factors must be primarily responsible e.g. a loss of cellular H^+ to the plasma.

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Disturbances of Hydrogen Ion Balance Occurring in Premature Infants

II *Late Metabolic Acidosis*

by POUL KILDEBERG

Testing selected functions of the infantile kidney and using several different bases of comparison, a number of authors have shown renal performance in the newborn infant to be "immature" and in several respects inefficient as compared with that of adult individuals [2 19]. As to the neutrality regulating functions of the premature kidney the available evidence is limited. In their study Tudvad *et al* [20] found the maximum rate of bicarbonate reabsorption in healthy premature infants to be comparable to that of adults. Gordon *et al* [5] from ammonium chloride tolerance experiments, concluded that the renal production of ammonia is deficient in premature infants, while Rubin *et al* [17] by feeding $CaCl_2$ to a group of prematures demonstrated such a deficiency only in the smaller infants (appr 1500 g) The relatively small amount of titratable acid excreted in the urine of newborn infants appears to be related to the amount of buffer substance excreted rather than to a deficient secretion of H [14].

However as pointed out by McCance & Widdowson [13], the results of such studies should be considered in the light of the general homeostatic mechanisms i.e related to the job the kidneys actually have to do, and attention has been drawn by the same authors to the significance in this respect of the particular anabolic situation of the infantile organism. Thus, another and possibly more physiologic approach to the evaluation of renal functioning in the newborn infant which also takes into account the functional variability often observed in prematures, would consist in searching such infants for signs of renal inadequacies (in terms of adult standards) and next solving the more difficult problem of assessing the physiologic or pathologic significance of such inadequacies, i.e establishing true normal standards for renal performance in infancy

In the present paper a type of late metabolic acidosis occurring in some premature infants will be described and examined with a view to the possible role played by the renal tubular functions in the production of the disturbance

TABLE 1 *Clinical data of 12 premature infants with late primary metabolic acidosis.*

Oral feedings have been initiated 24–48 hours after birth. From the beginning of the second week, the formula in question has been administered in an amount corresponding to 200 g/kg body weight/24 hours.

N	Sex	Weight (in g) At birth	7th day	14th day	Diet	Delivery and neonatal period	Minimum base excess recorded (Age in days)
1	♀	1700	1650	1620	Mamyson®	Normal	−9.0 mEq/l (7)
2	♂	1700	1700	1800	—	Meconium-stained amniotic fluid. N extra-uterine asphyxia	−9.5 — (10)
3	♂	1800	1600	1700	—	Normal	−11.1 — (12)
4	♂	1800	1825	1830	CM + W (50 %)	Normal	−7.2 — (22)
5	♀	1850	?	1820	Mamyson®	Breech delivery. Serum bilirubin concentration on the 5th day 20 mg/100 ml	−21.8 — (17)
6	♀	2100	1980	2065	—	Normal	−8.9 — (16)
7	♀	2300	2270	2400	—	Normal delivery. Short convulsive episode on the 2nd day	−8.0 — (7)
8	♂	2300	2170	2150	CM + W (50 %)	Slight transient cyanosis	−10.5 — (13)
9	♂	2300	2100	2165	Mamyson®	Slight intra-uterine asphyxia. Breech delivery. The infant appeared healthy	−7.0 — (11)
10	♂	2370	2300	2300	CM + W (50 %)	Delivery normal. Mild respiratory distress	−15.2 — (17)
11	♂	2575	2370	2400	—	Normal	−14.5 — (19)
12	♀	1850	?	?	Pelargon®	Normal. (Breech delivery)	−20.0 — (42)

See Table . CM + W = Cow milk in water

Methods and Case Material

This study is based upon 482 determinations of the acid-base status made in 128 premature infants as well as supplementary measurements of the urinary net acid excretion. A general description of the case material has been presented in a previous report [1.] concerning different types of acidosis occurring shortly after premature birth. Further details are contained in Tables 1 and 2 of the present paper.

The acid base measurements were carried out using the Astrup micro equipment [1 18].

The urinary net acid excretion (NAE) may be defined by the equation

$$NAE = TA + NH^{+}$$

where TA denotes titratable acid obtained on titrating the urine to end point pH 7.40 at pCO 0 mm Hg—i.e. TA can be interpreted as H^+ excreted—in excess of the equivalent of the bicarbonate content of an equal volume of normal glomerular filtrate (.5 mEq/l)—in combination with the volatile and nonvolatile buffers of the actual urine. NH_4 signifies H^+ excreted in combination with ammonia.

Twenty four-hour urine specimens were collected through glass funnel attached to the penis. A few crystals of thymol were added to the urines which were titrated within two hours after end of collection. NAE was

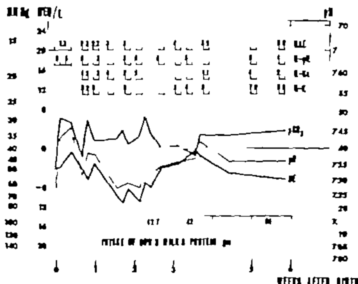


Fig. 1 Course of acid-base parameters in premature male infant with late primary metabolic acidosis (Table 1 no. 3). NAE = Net acid excretion (mEq). Urine N and urine-K are in mEq. BE = Base excess (mEq/l).

measured by the method of Jorgensen [9] using the Radiometer automatic titrator TTT 1

Results

In eleven prematures (Table 1)—8 6 % of the infants studied—a special type of metabolic acidosis was demonstrated, which from both clinical and laboratory criteria was easily distinguishable from the neonatal acidoses described previously [1-]. The disturbance presented as a hyperchloremic metabolic acidosis which developed gradually during the second and third weeks of life and usually did not reach severe degrees (Fig 1 and 2) Spontaneous remission as a rule set in within a couple of weeks. Respiratory compensation often but not always present did never reach the degree of efficiency achieved by older infants and children with metabolic acidosis [11]

In several instances, the diagnosis of metabolic acidosis came rather unexpectedly in apparently healthy infants showing no outward signs of illness. Only later did a review of the clinical records disclose retarded initiation of weight gain and, in some cases slow and indolent drinking at the height of the acidosis as clinical characteristics of this group

Incidentally none of these infants were asphyctic at birth. In five infants the perinatal course was completely uneventful, while minor difficulties were observed in the remaining six (Table 1) Hence this acidosis did not appear to reflect particular happenings during the perinatal period nor were these prematures particularly small at birth the mean birth weight being 2081 g

For this group a mean weight gain of −58 6 g (range −275 – +100) was computed for the first 14 days of life Pro-

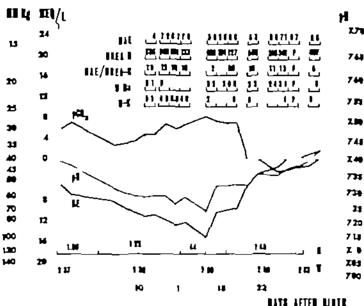


Fig. 2. Course of acid-base parameters in premature male infant with late primary metabolic acidosis (Table 1 no. 10). NAE = Net acid excretion (mEq). Urea-N = Urea nitrogen excreted (mg). NAE/Urea-N is in mEq/g. Urine-Na and urine-K are in mEq. N = Nitrogen of diet (g/day). W = Body weight (kg).

tracted initial weight loss was not conspicuous, the low 14th day weight score being mainly due to a delayed start of weight gain. As controls were used 20 consecutive cases fulfilling the following requirements: A normal perinatal clinical course, a normal acid base status as judged from the existing measurements, and artificial feeding from birth. The mean birth weight of these control infants was 1702 g and the mean weight gain from birth to the 14th day of life +166.8 g (range +60 – +460). Thus the development or non-development of this late metabolic acidosis appeared to be associated with the weight response to the early feedings which in the infants in whom acidosis subsequently developed suggests that less nitrogen was retained during the pre-acidotic period.

In one infant (Fig. 3) the acidosis was unusually severe (minimum pH 7.05 at BE −24.8 mEq/l) but following a few oral doses of bicarbonate it disappeared completely. This would seem to suggest that the acidosis was due to an acid load accumulated during the first weeks of life, of which the infant was not yet able to rid herself, but not due to a continuous process still active at the time of treatment,—or alternatively that a vicious circle was broken. The acidosis was discovered by a routine determination of the acid base status, the infant appearing quite vigorous throughout the acidotic period.

The development of this type of acidosis at a time (2nd–3rd week) when the daily protein intake usually reaches a temporary maximum, as well as absence of diarrhea, dehydration, oliguria or other outward signs of illness in these cases, suggested

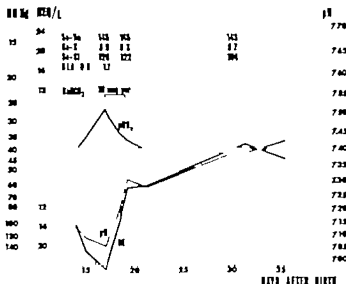


Fig. 2. Course of acid-base parameters in premature female infant with lat primary metabolic acidosis (Table 1 no. 5). BLA Blood lactic acid. Blood values are in mEq/l.

that a disproportion between the daily net load of non-volatile acid and the renal capacity for hydrogen ion excretion during the first few weeks of life was the direct cause of the disturbance

An equally satisfactory response to a single infusion of bicarbonate and a simultaneous change of diet was observed in a small premature (Table 1 no 12) who had for four weeks been maintained on a Pelargon[4] diet containing in the strength used, cow s milk protein 2.2 % and added (racemose) lactic acid, 31 mEq/l, but not thrived well. This patient presented with slight dyspepsia and a BE value of −20.0 mEq/l. Overloading with protein and lactic acid [4] appeared a reasonable explanation of the acidosis in this case

In order to get an estimate of the NAE resulting from feeding ordinary cow s milk preparations to healthy premature infants with a normal and approximately constant BE, the data of Table 2 were collected. Furthermore two young healthy adults (weight 58 and 67 kg) were for a four-day period placed on a diet containing a daily supply of 2–2.5 liters of cow s milk and additional calories in the form of pure sucrose The urea nitrogen excreted amounted to 94 % of the nitrogen ingested, the overall NAE value being 4 75 mEq/g of nitrogen ingested, 5 00 mEq/g urea N excreted (range 4.0–6._) or 0.8 mEq/g protein converted to urea The larger amounts of milk titratable acid expressed per g of urea-N excreted demanding oxygenative or renal removal in infants with a positive nitrogen balance intestinal production of lactic acid and a greater tendency to urinary excretion of lactate in the infants may contribute to the observed difference between the adult figures and those of Table 2, cf discussion p 522. An

attempt will be made to match the NAE values of Fig 1 and 2 with estimates of the corresponding net acid production (NAP) based upon these figures (p 524)

Complicating infections and other disorders indirectly related to prematurity may give rise to other acid base disturbances more similar to those encountered in later life During the present study infectious gastroenteritis accompanied by extraordinarily low BE values was found in some instances in 1–3-week-old prematures. As in the case of primary late metabolic acidosis respiratory compensation was often relatively poor

The lowest BE recorded, −30.5 mEq/l was observed in a 16-day-old premature girl transferred from the nursery of the Obstetric Department because of diarrhea started two days before Pregnancy and delivery had been uncomplicated. The birth weight wa 1850 g. The infant cried immediat ly and ppeared healthy during the first two weeks, apart from anemia. On the 3rd day a hemoglobin value of 14.8 g/100 ml was obtained. On the 14th day the hemoglobin concentration was unchanged and the weight 1950 g On admission to the Pediatric Department the infant appeared pale and slightly jaundiced. The stools were yellowish, liquid but apparently not infectious. The acid base status showed pH 6.91 BE −30.5 mEq/l and pCO_2 16 mm Hg (total CO_2 3.5 mEq/l)

In spite of treatment with fl id, bicarbonate antibiotics, and hydrocortisone the infant died 24 hours after admission. The macroscopic post mortem examination revealed no abnormalities. A histologic study of the kidneys showed normal glomeruli and normal tubular cells. In the proximal sections of the collecting ducts luminal precipitates of a homogenous eosinophilic material were noted, which did not resemble hemoglobin. More distally calcification of the precipitates became prominent The acidosis was thought to be of renal origin.

Discussion

The gradual development of the primary late metabolic acidosis in apparently healthy prematures displaying no signs of specific illness points to a disproportion between the daily NAP and the renal capacity for hydrogen ion excretion during the first weeks of life as the most likely single cause of this type of acidosis Such disproportion might come into existence in a number of ways. (1) Specific renal tubular insufficiency relative to a "normal" NAP (2) Abnormally" increased NAP relative to a 'normal renal capacity for H^+ excretion (3) A combination of both. An abnormally large input of non volatile acid, in infants receiving ordinary cow s milk s feedings, might result from a low nitrogen retention or/and increased urinary loss of lactate due to the use of 'lactic acid milk (4) increased production of lactic acid through intestinal fermentative decomposition of lactose in complete oxygenation of lactate by the tissues [6], or insufficient tubular reabsorption of filtered lactate The present data permit of no complete evaluation of these possibilities but throw some light on some of them.

Owing to a pH somewhat below 7 0 and its content of buffer substances, milk—whether human or cow's milk—has a definite titratable acidity However because the milk titratable acid rather closely equals the amount of lactate present in the milk it does not charge the renal mechanisms provided that all combustible milk buffer base (chiefly lactate) is properly oxygenated by the tissues If lactate is excreted in the urine the equivalent amount of H^+ will have to be disposed of by the kidneys.

TABLE 2. *Net acid excretion (NAE) in eight healthy infants and one infant with mild respiratory acidosis*

Expressed per g of urea-N excreted the NAE values become rather uniform, the high values of infant no. 2 being probably due to increased renal reabsorption of bicarbonate in response to the elevated pCO_2.

No.	Birth weight (g)	Weight at sampling (g)	Age at sampling (days)	50 % cow milk and 5 % sucrose in water(*) (ml)	Mamysan (*) (ml)	Breast milk (ml)	(Urea-N excreted)/N of diet 100 (%)	BE (mEq/l)	pCO_2 (mm Hg)	Diuresis (ml)	NAE (mEq/24 hours)	NAE/N of diet (mEq/g)	NAE/Urea N excreted (mEq/g)
1	1200	2140	52–53		460		38	2.1	38	100	5.2	2.8	8.9
2	1600	1780	20–21		370		10	+1.8	57	170	1.2	1.3	12.5
—	—	2030	29–30		400		31	−0.4	54	40	4.0	2.7	12.0
3	1800	2200	17–18		400		18	+1.2	38	155	1.8	1.3	5.2
—	—	2300	25–26	420			37	1.8	41	245	2.9	2.4	9.0
4	1600	2240	49–50	200	340		47	0.0	35	250	6.0	4.7	8.2
—	—	2360	60–61	200	240		45	−2.1	33	275	6.9	5.1	8.2
5	2200	2040	6–8			330	15	−2.2	39	165	3.0	1.3	8.8
6	2700	2870	16–17		480		40	+0.8	42	215	4.5	3.1	0.8
7	2350	4000	56–57	700			49	+2.0	47	440	5.2	2.9	5.9
8	3400	4100	57–58	780			8	+1.2	41	580	8.7	4.0	5.9
9	3700	3800	18–19	900	400		43	−0.2	40	480	7.2	3.6	5.7

Titratable acid about 4 mEq/l.
* Powdered cow milk preparation imitating human milk. The 14 % solution used contains 2.0 % protein and has a titratable acidity of about 6 mEq/l.

In order to evaluate accurately the potential acidity of any particular milk diet in terms of the net load of H+ demanding renal excretion in any particular infant, the known quantities should include the amount of combustible milk buffer base passed into the urine the fraction of dietary nitrogen appearing as urea-N and the amount of non-volatile acid released by the conversion of the milk proteins to urea.

However comparing the NAE values corresponding to the pre-acidotic period of Fig 1 with those of Table 2 shows that while the daily protein load could probably not explain the accumulation, in this patient of non volatile acid in the presence of a strongly positive nitrogen balance it might possibly do so in the case of a zero balance At the height of the acidosis the NAE values are quite comparable to those of Table 2 and, like those of Fig 2 tend to vary proportionately rather than inversely as the BE

The data of Fig 2 include the urinary of urea N In spite of failing weight gain during the first weeks of life this infant retained about 50 % of the nitrogen administered as cow s milk s protein during this period and it is seen that the NAE expressed per g of urea-N excreted clearly exceeds that found in normal infants. In this infant the BE decreased steadily from the 10th to the 16th day in spite of apparently abundant renal NAE and absence of external losses of base After replacement of the milk feedings by 10 % glucose in water for two days, resumption of the protein intake did not compromise the gradual rise in BE, and restoration of extracellular neutrality was followed by a drop of the NAE to the level found in the two adult individuals.

It appears, then, that renal incapability to handle the amount of non volatile acid "normally excreted in the urine of premature infants receiving a corresponding diet is probably not the cause of this primary late metabolic acidosis, while it seems quite possible that these acidotic infants have been facing larger loads of nonvolatile acid than have the normal control infants (cf p 522) This would correspond well with the relative inefficiency of the premature kidney to deal with induced acidosis demonstrated by several authors [3 4, 5 7 17] and the observation during the present study of unusually severe secondary acidosis in premature infants aged 1–3 weeks.

Possibly the premature substitution of extrauterine conditions including acidogenic enteral feedings for the maternal environment and placental homeostatic functions requires processes of enzymatic adaptation to take place in the tubular cells. The self limiting character of the late metabolic acidosis may indicate a process of substrate maturation i.e. the acidotic stress on the kidneys may constitute a prerequisite to the proper progress of such adaptation.

As recognized by Tudvad *et al.* [*0] the demonstration, in premature infants of a capacity for renal tubular hydrogen ion *secretion* i.e a maximum rate of tubular bicarbonate reabsorption in an actually alkalotic state (basic urine) comparable to that of adults does not necessarily imply a corresponding capacity for the *excretion* of a positive NAE during the stress of acidosis, the latter depending primarily upon the rate of ammonia for

mation, the filtered amount of buffers and the maximum hydrogen ion gradient established across the luminal tubular cell membrane

As a further contribution to the development of the late extracellular metabolic acidosis one might consider th possibility of a loss of cellular H^+ to the extracellular fluid. The steady decrease in sodium excretion and corresponding rise in potassium excretion shown in Fig 1 and also recognizable in Fig _, might indicate increasing adrenocortical activity That *temporary adrenocortical hypofunction* may occur rather frequently in newborn infants was, in fact suggested by Jaudon [8] and Provenzano [16], but at the present time, a possible relationship of adrenocortical hypofunction to an internal redistribution of H^+ can not be defined As shown by Pitts [15] in experiments with rats, adrenal insufficiency compromises the renal response to an acid load.

Summarizing, it appears that the metabolic acid base status during the second and following weeks of life reflects the integrated effects of several factors including the potential acidity of the diet, the degree of protein catabolism the functional status of the renal tubules, and probably endocrine homeostatic functions and that in prematures who do not thrive appreciably during the first weeks the net effect is often a metabolic acidosis. That the balance achieved may be a very subtle one is indicated by a prompt response to simple therapeutic measures (Fig and 3) Failure to thrive more or less increases the charge of the kidneys but possibly in addition, bears some relationship to other factors, e.g the neonatal adrenocortical status.

The weight loss of the infants with late metabolic acidosis was mainly confined to the first week of life whereas the acidotic trend did not become apparent until later which leaves little doubt that the failure to thrive was a primary factor Obviously the increasing acidosis may further adjourn the start of weight gain [10] and a vicious circle result. It is common clinical experience that many otherwise healthy prematures do not thrive appreciably during the first one or two weeks of life It would appear that in such cases the possibility of metabolic acidosis should be *borne in mind, and that with low BE* values the dietary protein load should be reduced rather than increased.

Summary

A type of late (2nd–4th week) metabolic acidosis occurring in apparently healthy prematures receiving ordinary cow s milk s feedings (not enriched with protein or lactic acid) is described. It appears that delayed start of post natal weight gain is a primary factor in the genesis of this disturbance which was demonstrated in 8 0% of 123 premature infants.

The acidosis is probably caused by a temporary disproportion between the renal capacity for hydrogen ion excretion and the daily load of non-volatile acid which in these infants may be larger due to low nitrogen retention and increased urinary loss of lactate Other factors may be partly responsible

The acidosis may be removed by means of a few doses of bicarbonate or a temporary withdrawal of the milk diet In prematures who do not thrive appreciably during the first weeks of life the possibility of metabolic acidosis should be borne in mind

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Hereditary Pituitary Dwarfism Treated with Human Growth Hormone

by O TRYGSTAD and M. SEIP

The role of genetic factors in the etiology of pituitary dwarfism is still a matter of dispute Daughaday [4] maintains that "in contrast with the pituitary dwarfism of mice the human disease does not appear to have a hereditary basis However in another chapter of the very same textbook Motulsky [9] accepts that the condition occasionally may be due to utosomal recessive inheritance

Previous literature on the subject has recently been reviewed by Grebe [5] and by Nowakowski & Lenz [10] But the un certainty of the clinical diagnosis of pitui tary dwarfism in many of the reported familial cases makes the evaluation of genetic factors difficult However there are also some recent reports, where the diagnosis has probably been more firmly established [2, 10 14] although few de tails are given.

In the following discussion a family with a high incidence of dwarfism will be presented. Three members of this family (two of whom are siblings) have been studied in detail. The diagnosis, pituitary dwarfism has been confirmed, and all three patients have been successfully treated with human growth hormone.

Case Reports

Case 1

A. S. is a girl born March 29 1949 Three older siblings are f normal height but a younger brother (Case) suffers from pitui tary dwarfism. Many relatives, both paternal and maternal, have been dwarfed or of short st ture (Fig 1). The father measures 185 cm the mother 152 cm.

The patient was born at term following normal pregnancy and delivery Birth weight and length were normal, but already during the first year of life growth retardation became obvious. Her psychomotor development was normal, and her progress in school has been erage. At seven years of age she measured only 87 cm. Since then she has been growing less than 2 cm per year

On admission t the Pediatric Depart ment, Uni ersity Hospital, Oslo Nov 24 1961 her height was 96 cm, i.e 45 cm below the 2.5 percentile. N pubertal development was present. The clinical findings and laboratory dat (T bles I and 2) were consistent w th the diagnosis of pituitary dwarfism, with reduced formation of somatotropic, gonadotropic thyreotropic and adrenocorticotropic hormones.

Treatment with human growth hormone 2 mg three times weekly and 1 thyroxine 0.1 mg daily was initiated Dec 1961 During the first year of treatment she grew 11 cm, and during the second year 8 cm.

TABLE 1 *Clinical and laboratory data*

Case No.	Sex	Age yrs.	Height age yrs.	Skeletal age yrs	PBI γ %	Cholesterol mg %	Phosphorus mg %	Phosphatase Bodansky U	Insulin sensitivity
1	F	13	3	7	4.5	290	4.2	[illegible]	Increased
2	M	10	2	2	2.6	237	3.8	1.8	Increased
3	F	19	11	16	2.7	304	3.8	6.8	Increased

Case 2

C S. is a younger brother of the first patient, born April 8 195 (Fig. 1) He was born at term following normal pregnancy and delivery Birth weight and length were within normal limit Growth retardation was observed already during the first year of lif His psychomotor development has been slightly retarded, and he started in school one year delayed. His growth has been very much retarded From 1957 his average yearly height increment has been 1.5 cm.

On admission to our department Nov 24 1961 he measured only 85.5 cm, i.e. 40 cm below the ..5 percentile. In contrast to his sister he exhibited light, but obvious clinical signs of secondary hypothyroidism. The clinical findings and laboratory data confirmed the diagnosis, pituitary dwarfism (Tables 1 and 2)

He was treated with human growth hormone mg three times weekly and l-thyroxine 0 1 mg daily from Dec 1961 During the first year of treatment he grew 13 cm, and during the second year 8 cm.

Case 3

R. E. is a woman born May 28, 1944 second cousin of the two foregoing patients (Fig. 1) Her mother measures 154 cm and her father more than 170 cm. T o younger sisters are of normal height. Her birth weight and length were normal, and she seemed t grow normally during the first two or three years of life although no exact measurements are available from this period. From then on growth retardation was observed. Her yearly height increment was about 3 cm until she was 18 years of age since then about * cm.

She was admitted to our department in September 1963. No signs of sexual development were present Chromosome studies revealed nothing abnormal. The diagnosis pi tuitary dwarfism was confirmed (T bles 1 and 2) Treatment with human growth hor mone 3 mg three times a week nd l thyrox ine 0 1 mg daily was begun Since then she increased 3 cm in height in less than three months.

TABLE 2 *SU-4885 test*

Case No.	17 KS (mg) Day 1	17 KS (mg) Day 2	17 KS (mg) Day 3	17 KGS (mg) Day 1	17 KGS (mg) Day 2	17 KGS (mg) Day 3	THS (μg) Day 1	THS (μg) Day 2	THS (μg) Day 3
1	0.8	0.4	1 0	3.2	1 9	3.7	0	162	134
				2.8	0.8	1 0			
3	0.5	0.8	1 3	4.2	4.2	7 8	60	440	7100

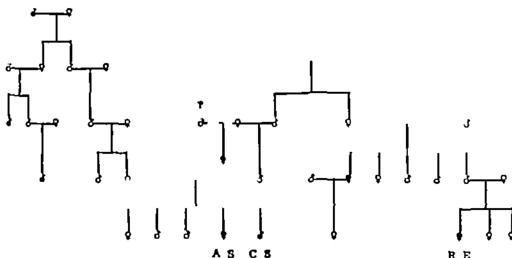


Fig 1 Pedigree of the reported family Black signs indicat abnormally short individuals, whit signs individuals of normal stature. Initials indicat the three cases of proven pituitary dwarfism

Discussion

Two siblings, brother and sister with severe pituitary dwarfism have been stu died. From early infancy they showed marked retardation of growth. At 13 years of age the girl s height was no more than the 3 year norm. The boy's height was only two years when his chronological age was 10

It has often been postulated that the growth rate in pituitary dwarfs is normal or approximately normal during the first two or three years of life However this is not always the case In our two siblings growth retardation was obvious already during the first year of life We have been able to make similar observa tions in several other patients with hypopituitarism

Another interesting observation is the high incidence of dwarfism or stunted growth in this family both on the paternal and maternal sid (Fig 1) The third case reported is a paternal second cousin of the two siblings. She was studied when she was 19 years of age and measured 14 cm

All these three patients showed signs of decreased formation of somatotropic, thyreotropic and corticotropic hormones (Tables 1 and) and will probably also be hypogonadotropic. No sexual development was present in any of them when last observed at 19 15 and 12 years of age The urinary excretion of corticosteroids (Table 2) was low in all three The response to Metapyrone (SU-4885) was poor in the two siblings, while in their cousin there was a normal increase in THS but a relatively small rise in 17 KGS

Treatment with human growth hormone has been highly effective so far The two siblings increased 11 and 13 cm in height during the first year of treatment and 8 cm during the second. The third patient grew 3 cm in less than three months. Some of the hormone used was prod ced in Uppsala in Gemzell laboratory some of

it in our own laboratory by the method described by Roos, Fevold & Gemzell [13]. In addition to human growth hormone three times weekly a small daily dose of thyroxine was given. This, of course is recommended when signs of secondary hypothyroidism are present and is probably advantageous in most hypopituitary dwarfs under treatment with somatotropin because an adequate supply of thyroid hormone is required to obtain optimal growth.

In addition to the three reported patients, five other members of the family (three males and two females) through five generations are known to have been abnormally short with adult heights decidedly below 150 cm (Fig. 1). These members have not been studied in detail and we therefore cannot maintain with certainty that the short stature has been due to hypopituitarism in all of them, although this may be a reasonable assumption. *A genetic basis for the dwarfism may* be presupposed. The two dwarfed siblings A. S. and C. S. have three paternal and three maternal relatives with adult heights of less than 150 cm, a fact which may perhaps explain their extreme degree of dwarfism.

The mode of inheritance in familial cases of pituitary dwarfism has been postulated to be autosomal recessive [5, 9, 10]. This coincides with our observations in the reported family. Whether the hormonal deficiency in these cases is due to an inborn error of metabolism similar to e.g. that in the adrenogenital syndrome or in the hereditary types of hypothyroidism is not known. It might be mentioned that pituitary dwarfism of hereditary origin is well known in mice [3].

Two of the family members *with stunted* growth have married and produced children (Fig. 1). It may be claimed that this is evidence against the contention that short stature in these two cases was due to hypopituitarism. However patients with pituitary dwarfism and normal sexual development have been described [1, 10]. Nowakowski & Lenz [10] maintain that in the hereditary type reduced production of somatotropic hormone without concomitant deficiency of gonadotropic hormones is more frequent than in sporadic cases. The two family members in question may be examples of such isolated growth hormone deficiency. Among other patients with pituitary dwarfism we have studied there are at least two, in whose families there is a remarkable incidence of individuals with stunted growth and normal sexual development. Particularly in such families the possibility of discovering individuals with isolated growth hormone *deficiency should* be considered.

In this connection it is interesting that cases of familial and hereditary hypogonadotropic hypogonadism without dwarfism have been described repeatedly [–, 7, 8]. Several other hypothalamic hypophyseal disturbances may also be genetically determined, e.g. diabetes insipidus, the Laurence–Moon–Biedl syndrome and Albright's syndrome. In the familial cases of generalized lipodystrophy described by Seip & Trygstad [15], a hereditary disturbance of the *hypothalamic* hypophyseal system is probably also present. Even acromegaly may in rare instances show familial occurrence [0].

In most cases of hypopituitarism all or several pituitary hormones are deficient although often to a variable degree. Some-

times, however one hormone is exclusively or almost exclusively affected. In addition to the isolated deficiencies of somatotropic, antidiuretic or gonadotropic hormone mentioned above an isolated lack of adrenocorticotropic hormone has also been described [11] Possibly the other pituitary hormones may be similarly affected

The frequency of the isolated lack of somatotropic hormone may not be constituted, until reliable methods to determine the growth hormone level in the blood are more readily available Probably a certain number of patients classified today as primordial dwarfs belong to this category In fact Raben [12] in a few cases of socalled primordial dwarfism has obtained good results by treatment with human growth hormone in the usual dosage

Summary

A family with high incidence of dwarfism and stunted growth is presented Eight members of the family (four males and four females) through five generations are known to have been abnormally short Three of them, two of whom are brother and sister have been studied in detail. The diagnosis pituitary dwarfism has been confirmed and treatment with human growth hormone has been effective The disease is thought to have a hereditary basis in this family probably with autosomal recessive mode of transmission

It is pointed out that some patients with pituitary dwarfism show obvious growth retardation as early as during the first year of life The possibility of discovering individuals with isolated growth hormone deficiency (without concomitant lack of gonadotropic hormones) is discussed Other hypothalamic hypophyseal diseases caused by genetic mechanisms are mentioned briefly

Acknowledgement

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Measles Vaccination

II Antibody Titers 8 to 9 Months after Vaccination with an Inactivated Vaccine[1]

by E. NORRBY GUN CARLSTRÖM, R. LAGERCRANTZ and S GARD

A number of field trials with inactivated measles vaccine have been reported [1 –, 3, 4 5 9]. Serologic conversion rates of 90 % were observed, protective effects of about 92–96 % against typical measles and 80–68 % against cases of all degrees of severity and lasting for at least several months after vaccination were recorded. However the serologic responses were usually of comparatively short duration and circulating antibodies were often no longer demonstrable 3 to 8 months after vaccination [–, 9].

In a previous paper [7] the present authors reported results of a small scale field trial with formalin-inactivated, alum-precipitated measles vaccine prepared from monkey kidney tissue culture-grown virus. The present paper describes serologic follow up studies of the vaccinees

Material and Methods

Study population Sixtyseven hildren aged 6 t 4 months at the time of vaccination participated in the trial. As previously described they received two or three monthly inoculations of vaccine supplied by Pfizer Inc. Pre and post vaccination blood samples were collected and tested as previously described.

For f llow-up studies 57 of the 67 children were available. Blood samples were collected 8 to 9 months after the last inoculation, 0.2 ml of blood was drawn from the finger tip and immediat ly mixed with 0 6 ml of tissue culture medium containing heparin in a concentration of 1 –5,000. After centrifuga tion the supernat was collected and inacti vated for 30 minutes at 56°C The specimens were considered to represent a 1 5 serum dilution.

Interviews concerning possible exposure to measles and its outcome were performed at the time of bleeding and again 8 months later

Neutralization tests In order to enhance the sensitivity of the test the antigen was prepared as follows. Monolayer cultures of a human embryonic cell line (strain Lu 106) were noculated with the Edmonston strain of measles virus. When a clearcut cytopathic ffect appeared med um was changed and the cultures were harvested after further two hours incubation t 37°C Material thu prepared exhibited infectivity titers of about 10^6 TCID/ml with comparatively low hemagglutinin (HA) content, indicating a f vourably small proportion of non infective ant body binding antigen. Tests were set up

[1] Supported by grants from the Swedish Medi cal Research Council.

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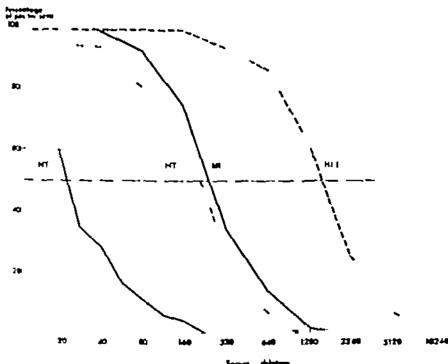


Fig. 1 Immunologic "profiles within group ( ). Accumulated neutralization (NT I and II) and HI (I and II) titer distributions of 10–14 day (I; dashed lines) and 8–9 month post vaccination serum samples (II; full lines). d. on the bacisse stands for delayed break through of virus at low serum dilutions.

Comparison between 10–14-day and 8–9-month postvaccination results is not strictly permissible as simultaneous tests on paired sera were not feasible In parti cular the neutralization titers have to be judged with caution, as the technique for preparation of antigen as well as the antigen dose used in the test were not the same on the two occasions Reference sera were not included in the tests either

Thus, the comparative ratios calculated in the table are presented with these reservations. The ratio of median HI to neutralization titers on the first occasion 7.3, was increased to 11 This probably reflects the increase in antigen dose from 40 to 100 TCID in the neutralization test. The average reduction in titer over the 8 to 9 months amounts to 6 4 times in the HI test which for reasons already indicated must be considered as the most reliable indicator Therefore since the lowest serum concentration tested in the CF test was 1 20 and with some sera 1 40 it is obvious that no positive results were to be xpected in this test.

The shape of the immunity profiles" in Fig. 1 as well as the distribution of points in Figs. and 3 indicate that there i no systematic variation in the rates of reduction in titer Thus the correlation between late and early HI titers was calcu lated to +0 606 and the regression coef ficients (late on early titers and vice

TABLE I *Number of positive sera and mean titers in early and late post vaccination sera from children in group (a) The table also demonstrates the relative sensitivity of HI to neutralization test as well as change in serum titers with time expressed as the ratio between mean serum values in early and late post vaccination samples*

Time after vaccination for collection of sera		Serological test: Neutralization	HI	CF	Ratio between HI and neutralization titer
10–14 days	Frequency of positive sera Median titers	46/49 94% 1 220	48/49 98% 1 1600	36/49 73% 1 34	3
8–9 months	Frequency of positive sera Median titers	29/4 59% 1 23	48/49 98% 1 250	0/49 0% 1 10	11
Ratio between mean serum titer in the 10–14 day and 8 to 9 month blood sample.		9 6	6.4	—	

with an antigen dose of 100 $TCID_{50}$ and otherwise as previously described [7].

Hemagglutination-inhibition (HI) and complement fixation (CF) tests were carried out as previously described [7]. Prior to use in HI tests all sera were adsorbed with an equal volume of a 15% suspension of washed monkey erythrocytes. Kaolin treatment was omitted as unnecessary at the serum concentrations actually used in the tests.

All antigens used were derived from the same tissue culture system. Titer values recorded refer to the concentration of serum in the reaction mixture before addition of possible indicator systems. The lowest concentration tested was 1 10 in neutralization and most CF tests; 1 40 in HI and some CF tests. All sera were tested simultaneously in each one of the three serological reactions.

Results

In analysing and discussing the immediate results of vaccination we found it convenient to classify the vaccinees as follows.

a. Children without previous or intercurrent exposure to measles receiving three doses of vaccine

b Children receiving two doses only

c Children who were possibly exposed to natural measles in the course of vaccination.

d. One child with pre immunization antibodies indicating previous exposure

The follow up studies included 49 of 55 children in group a., 4 of 5 in group b. and 4 of 6 in group c

Serologic reactions The results of neutralization, HI and CF tests are summarized in Table I and Figs. 1 to 3. Of the children in group a, as shown in the table and in Fig 1 98 % had demonstrable HI antibodies 50 % gave clearcut positive neutralization tests, whereas none had demonstrable CF antibodies. With 1 sera break through in the neutralization test was considerably delayed, indicating subthreshold antibody titers (less than 1 10) These included the proportion of positive neutralization tests is increased to 84 %

immunity level only but on virus dose and other conditions as well. Secondly demonstration and assay of antibody is largely a matter of sensitivity of the methods used. The HI technique used in the present study and described in greater detail in previous papers [6 7] is apparently more sensitive than those applied in most other laboratories: titers observed are higher than those commonly reported in comparable cases and the presence of antibodies in our vaccinees was easily demonstrable 8 to 9 months after vaccination, i.e. at a time when in other studies mainly negative results were reported. Thus, inability to find antibodies does not necessarily mean that they are absent. Finally secondary immune responses evoked by contact with the virus in the course of the incubation period have to be considered, as well.

Under our assumption the problem of providing protection would then be equivalent to the question of maintaining an antibody titer above a certain level. The present material does not permit any estimates of such a possibly protective antibody titer. At th time of the 8 to 9 month blood sampling only three children had had known exposures to natural measles none of them showing any clinical signs of infection. In the interval between 8 to 9 months and 16 to 17 months after vaccination 4 children out of 11 probably exposed displayed definite symptoms. Although a trend may be hidden in these figures, it is obvious that no significance can be attached to them on account of the small numbers. Further observations on rates of reduction in titers and on the correlation of resistance to actual titers are obviously needed for clarification of this point.

Measles virus apparently carried at least two antigenically active structures the HA and the internal nucleoprotein [8]. The important question arises whether both these antigens are essential for production of protective antibodies. There is certain evidence in favor of the assumption that neutralizing and HI antibodies are identical [7 Norrby to be published] which would seem to indicate that the nucleic acid free hemagglutinin were responsible for the production of protective antibodies. Taking this cue we have planned a study of the prophylactic value of purified hemagglutinin as a vaccine.

Summary

Out of a group of 67 children vaccinated with an inactivated measles virus vaccine 57 were followed up by serological tests 8 to 9 months after vaccination. Among 49 children who had had the full course of three monthly inoculations and no known exposure to measles during the vaccination period 41 (84 %) had antibodies demonstrable in the neutralization test (highest serum concentration tested 1:20) and 48 (98 %) had HI titers of 1:40 or higher. As compared to the results obtained 10 to 14 days after vaccination antibody titers had declined on an average by a factor of 6 to 7.

In a period of 17 months after vaccination 15 children had experienced exposure to natural measles in siblings or close playmates. In 5 of those exposed clinical manifestations of measles were observed, 4 of these cases were classified as mild.

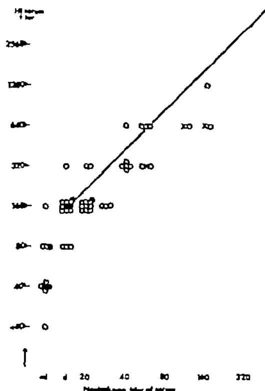


Fig. 4. Correlation between HI and neutralization titers of 8 to 9 month post-vaccination samples. d. stands for delayed break-through of neutralization antigen in low dilutions of serum. Symbols as in Fig. 2.

Discussion

In the discussion on living attenuated versus killed virus as a vaccine against measles it has sometimes been assumed that infection with live virus produces not only an immunity of longer duration, possibly lifelong, but also a more complete qualitatively superior immunity than does immunization with killed virus. This question most probably boils down to the following: Is serologic immunity alone sufficient or is some additional mechanism, for instance cell-associated immunity needed for protection against infection?

Present evidence to which our own observations may be added indicates that protection indeed is afforded by vaccination with killed virus. Thus it seems reasonable to assume that circulating antibodies are capable not only of modifying the course of an infection, which is a well established experience from the clinical use of immune gammaglobulin, but also of preventing infection.

If so the degree of protection should be correlated to the actual antibody titer. Those *observations reported* in the literature [1 3 4 5] when vaccinated children who no longer had demonstrable circulating antibodies yet remained symptomfree when challenged with wild or attenuated virus, do not necessarily refute this assumption. First of all the outcome of a challenge infection does not depend on the

TABLE 2. *Clinical data of children who displayed symtoms after natural exposure to measles*

Child	Group	Time of exposure in months after vaccination	Temperature Maximum C	Duration in days	Rash	Other clinical symptoms
J.-C.D.	( )	15	39.8	3	Faint	None
L.R.	(a)	14	38.1	1	Faint	None
T.W.	(a)	16	38.5	3	Faint	None
A.W.	( )	8	40.5	3	Regular	Cough, itching
A. K.L.	( )	12	39.9	3	None	Slight conjunctivitis

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The Changing Circulatory Pattern of the Newborn Infant Studied by the Indicator Dilution Technique

by W JEGIER,[1] W BLANKENSHIP [2] J LIND and A. KITCHIN[3]

Introduction

At birth the changes in the circulation from the intrauterine to the extrauterine type are not immediate but involve a transitional or neonatal period. In spite of all modern cardiological techniques used to analyze this transitional circulation its timing and the exact mechanism of its transition remains controversial.

Among the methods available the dye dilution technique for the study of the neonatal circulation particularly with respect to the time of closure of the ductus arteriosus was introduced by Prec *et l* in 19.5 This study was performed on a small number of infants using Evans blue as an indicator

The present study was designed to *amplify this investigation and to add a* study of the time-concentration components using indocyanine green as the indicator

Material and Methods

Eighty-nine full term, apparently normal infants from essentially uneventful pregnancies were studied. They ranged in age from 3 hours to 23 days. The mothers recei ed no anesthesia and the deliveries were vaginal without complications. One infant had talipes-equinovarus deformity one had a fractured clavicle and one had large cephalohematoma. In four infants a short, decrescendo, early systolic murmur was heard and recorded but this had disappeared by the seventh day of life in two f them. In the remaining two infants, in spit of the systolic murmur at the time of the last examination on the fifth and twenty-third day respectively apparently normal pattern indicator dilution curves were recorded. (See under results.)

The indicator used in this study was indocyanine green (manufactured in dry form by Hynson, Westcott & Dunning, Inc., Balti more Maryland). It was dissolved in an aqueous solvent to contain 2 mg/ml and the solution was used within 6 hours of mixing; 1.3 mg of the dissolved dye was used for each curv Three to five injections were per formed in each infant for a total dose not exceeding 5 mg/kg of body weight There were no toxic reactions. The indicator was injected and flushed with 1 to ml saline

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[2] Research Fellow Department of Pediatrics, Vanderbilt University School of Medicine Nashville, Tennessee, U.S.A.

[3] From Western General Hospital, Edinburgh, Scotland, sponsored for this project by council of Europe Medical Fellowship.

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Material and Methods

Eighty nine full term, pparently normal infant from essentially uneventful pregnancies were studied. They ranged in age from 3 hours to 23 days. The mothers received no anesthesia and the deliveries were vaginal without complications. One infant had a tall pes-equinovarus deformity one had a fractured clavicle and one had large cephalohematoma. In four infants a short decrescendo, early systolic murmur was heard and recorded but this had disappeared by the seventh day of lif in two of them. In the remaining two infants, in spit of the systolic murmur at the time of the last exa mination on the fifth and twenty-third day respectively apparentl normal pattern indicator dilution curves were recorded. (See under results.)

The indicator used in this study was indocyanine green (manufactured in dry form by Hynson, Westcott & Dunning, Inc Balti more Maryland). It was dissolved in an aqueous solvent to contain mg/ml and the solution was used within 6 hours of mixing; 1.8 mg f the dissolved dye was used for each curve Three to five injections were per formed in each infant for a total dose not exceeding 5 mg/kg of body weight There were no toxic reactions. The indicator was injected and flushed with 1 to ml saline

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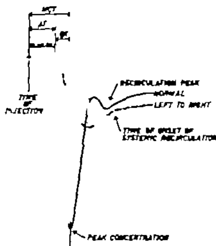


Fig. 1 Schematic representation of the normal and the left t right pattern f indicator dilution curves showing the time-concentration components.

into the jugular vein in 55 infants, the antecubital vein in 18 infants, a scalp vein in 14 infants and a dorsal hand vein in 3 infants. The injections were performed as rapidly as possible in one and one-half seconds or less, through a thin walled 21 gauge needle the flush being delivered from the side arm of a three way stop-cock.

The dilution curves were recorded by means of a photoelectric cell with a light source mounted on a spring clip and attached to the pinna f the ear The output of the cell was fed into a Cambridge electronic recorder which incorporated an impedance matching pre-amplifier system. The response of the system was approximately linear over the range of dye concentration used. Since indocyanine green has an absorption peak at 800 mμ an infrared sensitive photo cell with a peak sensitivity at approximately 800 mμ was used At this wave length, oxygenated and reduced hemoglobin have the same light absorption and thus any variations in the oxygen saturation of the arterial blood will not significantly affect the dye concentration curves [2*].

A time lapse of 5 to 10 minutes was allowed between the introduction of the needle and the recording of the curves. Only curves recorded from infants asleep or sucking quietly were included in the study and the ones showing excessive fluctuation of the base line or art facts due to movements of the infants were excluded. The infant were breathing room air and were one-half to two hours postprandial. A blood sample was drawn following the recording of the curves for the determination of venous hematocrit

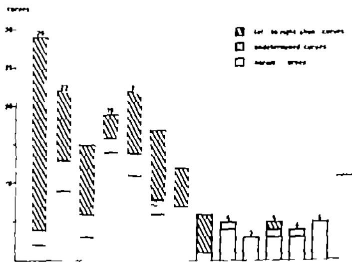


Fig. _ The curves obtained from infants on different days of lif Not lack f linear decrease in percentage of left t right hunt patterns with increasing age

TABLE 1 *The results of 175 curves obtained from 89 infants that were studied*

			No. of infants			
Age	No. of curves	No. of infants	Normal curves	Undet. curves	Shunt curves	Mixed* curves
1 d	29	17	1	1	15	
2 d	22	10	2		4	4 1 NSS 1 NS -UUN
3 d	15	6			3	3 1-USS 1-SNS 1-UUN
4 d	19	10	5		3	2 2-UN
5 d	22	9	3		3	3 1-US 1 NSSU 1-UN
6 d	17	9	3		4	2 1 NS 1 US
7 d	12	7	3		3	1 1-SN
8 d	6	2	1			
9 d	5	3	2	1		
10 d	3	1	1			
11 d	5	2	1			1 1 NUS
12 d	4	2	1			1 1 UN
13 d	5	2				
14-23 d	11	8	8			

* Mixed curves are infants with different patterns during the same investigational period
N Normal
S = Shunt type pattern
U Undetermined type of pattern

values. The blood was withdrawn into an heparinized syringe transferred to a capillary tube and centrifuged at 11,500 r.p.m. for 5 minutes after which the packed cell volume was read from the Guest chart [23]. One hundred and seventy five curves obtained from 89 infants were available for final analysis (Fig and Table 1) Of this group 16 babies were studied longitudinally up to 23 days (Fig 3).

Results

1 *Analysis of the types of curves*

The normal arterial indicator dilution curve following a single injection of indicator dye shows a time lag of a few seconds (AT) before the dye appears at the recording site This is followed by a build up period during which time the maximum concentration (MCT) is obtained. This peak concentration is followed by a rapid disappearance phase which follows an exponential course [9] The first appearance at the sampling site of dye which has traversed the systemic circulation for a second time causes the dilution curve to deviate from the exponential pattern and this is followed by a well defined systemic



Fig. 3. Group of eighteen infants with repeated investigation on different days of life. Two infants (numbers 9 and 24) had shunt and normal curves and two others (number 23 and 27) undetermined and normal curves recorded during the same investigational period.

recirculation peak (Fig. 4 and 1). The presence of an abnormal recirculation pathway through the pulmonary circulation, as in left to right shunts, alters the normal curve in two ways. The normal disappearance slope is interrupted by the arrival at the sampling site of dyed blood which has recirculated through the shunt pathway and the systemic recirculation peak is obscured by the abnormal recirculating blood. Small left to right shunts of less than 20% of the pulmonary blood flow give dilution curves which may be indistinguishable from normal [13]. As the size of the shunt increases the break in the disappearance slope comes earlier and is more marked. In large shunts the peak concentration of the curve which is re

Fig. 4. Dye dilution curves showing the change from a left to right shunt type pattern at 21 hours, an undetermined type pattern at 3 days and normal type pattern at 9 days of age.

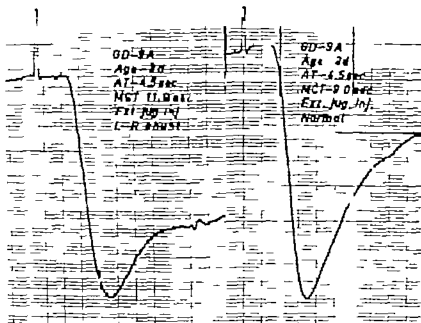


Fig 5. Indicator dilution curves showing variation in the type pattern during the same investigational period. This variation occurred without any apparent change in the stat of the infant.

duced because of dilution of the dye with the large flow of blood in the lungs, is followed by a prolonged disappearance phase in which the normal features of the disappearance slope and systemic recirculation peak are lost [13].

The classification of the curves obtained in this study has been based on the work of Carter *et al* [13]. The time-concentration curves were plotted semilogarithmically and the point of deviation of the disappearance slope from an exponential course was determined. The concentration of dye at this point (in mm deflection) was then expressed as a percentage of the peak concentration of the curve According to this calculated ratio, curves were then grouped into 3 categories The ones with ratios under 30 % were considered to have normal patterns and the ones with ratios over 35 % were considered to have left to right shunt patterns. The intermediate group was labeled undetermined" In curves showing a very large left to right shunt pattern, no definite point of the onset of recirculation is apparent in the disappearance slope These curves were included in the left to right shunt group

In six cases a variation in the type of curve recorded was noted on repeat recordings separated by 5 to 6 minute intervals (Fig 5) In 2 babies the change in pattern followed a period of crying In 4 infants the variation occurred without any apparent change in the stat of the infants None of the indicator dilution curves showed early appearance of the dye which would indicate the presence of a right to left shunt Table 1 summarizes the results btained from analysis of the curves. On

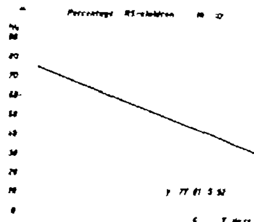


Fig 6 The percentage of infants with shunt type curves between the age of 1 to days. The points vary greatly around the line and analysis of the figure show that the variances bout the regression line are not constant and that the re gression coefficient does not vary significantl from zero. Thus there is no high probability that the percentage of hildren with shunt type curves does decrease in linear fashion with increasing age

the first day of 17 infants, 15 had shunt type curves one had a normal curve and one had an undetermined typo curve The following days of life show a rather wide scatter of values However after the 8th day only one shunt type curve was seen in 35 curves obtained from 18 infants. The percentage of infants with shunt type cur ves between the age of 1 to 7 days is shown in Fig 6 The points vary greatly around the line and analysis of the figure shows that the variances about the regression line are not constant and that the regression coefficient does not vary significantly from zero Thus there is no statistical probability to indicate that the percentage of children with hunt type curves does decrease in a linear fashion with increasing age

Fig 3 shows the result obtained from a group of eighteen infant studied longi tudinally at different ages It should be noted that the eighth day of life forms a dividing line between the occurrence of the shunt and normal type curves.

In Cases 7 9 10 and 28 an early decrescendo systolic murmur was heard and recorded over the precordial area In no other cases were murmurs detected. Phonocardiograms demonstrated the mur mur as occupying the first part of systole starting with the first sound and disappear ing in a decrescendo manner before the second heart sound Case 28 had no mur mur recorded on the first day of life and an "undetermined typo of curve was obtained On the second day the same systolic murmur was recorded and a nor mal dilution curve was obtained

From the above it follows that the change from shunt to normal type curves occurs at varying periods in neonatal life Our data are not complete enough to permit a statement as to the time of change from shunt to normal patterns in individual infants We can only point out that in four of the 18 infants that were repeatedly studied the normal pattern was recorded before the seventh day of life In the remaining 13 infant with the exception of Case 3 who had no follow-up after the seventh day of life the normal pattern appeared after the eighth day

2 *Analysis of the time compon t*

Four time components were selected for study

(*a*) The appearance time (AT) the time from the injection of the dye to the first detectable appearance of the indic tor at the recording site Fig 1

(*b*) The maximal concentrat on time (MCT) the time from the injection of th dye to peak concentration Fig 1

(c) The build up time (BT) the time between the first detectable dye arriving at the sampling site and the peak concen tration, i.e. MCT – AT BT Fig 1

(d) The time of onset of systemic recir culation (SRT) the time from injection of the indicator to the point of deviation of the normal curve from the exponential course due to systemic recirculation of the dye Fig 1

The AT and MCT were measured di rectly from the curves and tabulated for the normal type and the shunt type re spectively in Table 2. It was found that a statistically significant difference exists between the time concentration compo nents of the normal and the left to right shunt patterns ($P < 0.001$) This finding is in agreement with the data reported by Prec *et al* [34]. In order to determine the influence of the injection site on the time concentration components the groups were subdivided according to the venous site used for the injection of indicator In order to avoid statistical bias only the first curve of each infant was included in this evaluation. There was no significant difference in curves obtained following in jection into the jugular the antecubital

TABLE 2. *AT and MCT relation to type of d lution curves for all age groups*

The differences of AT and MCT in the normal and left t right shunt patterns is highl statis tically significant (P 0 0 1)

		Normal pattern	L R Shunt pa ern
Apperance time	N	45	41
	$\bar{x}$	4.60	6.03
	$s/\sqrt{n}$	0.124	0.205
Maximal conc. time	N	46	43
	$\bar{x}$	10.60	14.59
	$s/\sqrt{n}$	0._58	0.615

TABLE 3 *Appearance time and maximal concentration time n relation to type of dilution curves and injection sites for all ages*

X significant difference in time concentration components within normal and abnormal type group with different injection sites.

			Jugular vein	Cubital vein	Scalp vein
Apperance time	Normal patterns	N	29	11	5
		$\bar{x}$	4.75	4.77	5.10
		$s/\sqrt{n}$	0.137	0.354	0.557
	L–R Shunt patterns	N	4	11	8
		$\bar{x}$	3.96	6.11	6.15
		$s/\sqrt{n}$	0.314	0.616	0.712
Maximal conc. time	L–R Shunt patterns	N	24	11	8
		$\bar{x}$	12.83	15.82	15.10
		$s/\sqrt{n}$	0.567	1.547	1.671
	Normal patterns	N	30	11	5
		$\bar{x}$	10.57	10.74	10.46
		$s/\sqrt{n}$	0.314	0.616	0.712

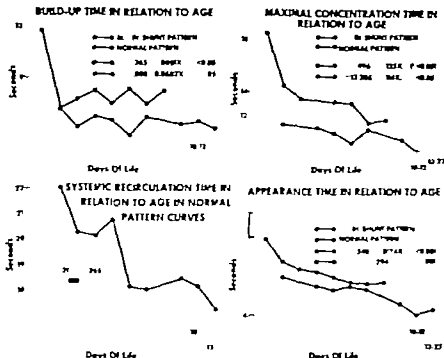


Fig. Time-concentration components (on the ordinat) plotted against -e in days (on the abscissa).

or a scalp vein, Table 3 although in the last group there were only 5 cases Thus it appears that the different injection sites did not change the time-concentration components significantly

Fig " demonstrates the relationship of different time-concentration components to the age of the infant. The values were measured on the individual dye dilution records and the mean value calculated for each day of life The values for the time of onset of systemic recirculation were obtained after the curves were replotted semilogarithmically Statistical evaluation showed that a significant decrease in the duration of the AT and the MCT occurs in the normal, as well as in the shunt type curves with increasing age of the infants. On the other hand, the BT which represents the difference between the MCT and AT seems to bear no relation to the age of the infants.

Discussion

The results of the present study suggest that most infants show *left to right shunt* type curves between the ages of 1- 4 hours which are rarely seen after the age of 8 days. In trying to pinpoint the site of this shunt one turns first to shunts known to exist during fetal life The foramen ovale seems a logical site for the occurrence of an intracardiac shunt James & Burnard [*6] using a platinum electrode in the right atrium and giving a breath of hydrogen gas to an apparently healthy infant concluded there was left to right shunting through the foramen ovale

during the first 24 hours, but not after that time In the new born lamb Barcroft *et al* [3] has furnished evidence that the foramen ovale closes immediately following the first breath From data presented by James [*7] a great majority of normal newborn infants have oxygen saturation values in excess of 92 % during the first 4 hours of life thus suggesting that a significant right to left shunt through the foramen ovale at rest probably does not exist The functional closure probably remains incomplete during the first few days of life and is closely related to change in intrathoracic pressure

The significance of the precapillary anastomoses between the bronchial and the pulmonary arteries in a normal neonate as described by Liebow [29] and their contribution to any left to right shunt in the new born period is at present unknown

Different opinions exist regarding closure of the ductus arteriosus and the direction of flow through it when patent Saling [30] suggests that the flow through the ductus arteriosus maintains its fetal direction (i.e. right to left) for as long as 9 hours after birth Eldridge & Hultgren [*1] found a significantly lower oxygen saturation of arterialized blood in the lower limbs as opposed to the upper limbs in infants under 3 days of age, but not after that age. They presumed that during the first 3 days of life a right to left shunt occurred at the level of the ductus arteriosus. James has reported an alternating flow during each cardiac cycle in a number of infants from 1 to 24 hours of age using angiocardiographic cineroentgen technique Adams & Lind [1] and James & Rowe [*7] have concluded from oxygen saturation data obtained at the time of cardiac catheterization that the ductus arteriosus may be patent for as long as 10 days From the data of Rudolph *et al* [38] it seems that of 7 infants under 34 hours of age only showed a left to right shunt using oxygen saturation data as a criterion They concluded "that the ductus arteriosus is functionally closed after 15 hours but since it is anatomically patent it may be opened under appropriate stimuli during the first 7 to 10 days of life Although their subjects were without evidence of cardiorespiratory disease 3 were mongols 1 was microcephalic, 3 were normal and 4 were infants born of diabetic mothers.

From Fig 5 it appears that the percentage of infants with left to right shunts patterns does not show a linear decline in the first 8 days of life This is further supported by Fig 3. The lack of a definite linear decrease of percentage shunt type curves with increasing age of the infants may suggest that the obliteration of the left to right shunt is not an irreversible event and that during the first weeks of life it may be an intermittent phenomenon. The fact that we were able to obtain different patterns from the same infant during the same investigational period would be in keeping with this hypothesis

The mean appearance time in infants during the first 4 hours of life is 7 0 seconds (S.E. 0 14) This value decreases gradually during the first 3 weeks of life and reaches a val e of 4.3 seconds (S.E. 0.21) at the age of 12 to 23 days This reduction in the AT is highly significant statistically ($P < 0.001$) The changes in the length of the AT were unrelated to the heart rate nor did they correlate with the hematocrit values.

The appearance time of dye particles at

the pick up site is governed mainly by the length of the pathway and the linear velocity of the blood flow through the segment of the circulation between the injection site and the recording site From data supplied by Kamaras [28] and others it is evident that major changes in the length of the pathway cannot be effective in producing the changes noted in the circulation time Also in this study the AT was not significantly affected by altering the injection sites

The explanation for the decreasing AT during the first week of life may in part lie in the known increase in the rate of volume flow in the peripheral circulation as demonstrated by Celander [14 15]. He has shown a significant increase during the first hours of life In infants under 12 hours of age the measured resting blood flow in the foot and calf was found to be in the region of 2 to 5 ml/min/100 ml and in infants between 1 and 30 hours of age the resting blood flow has increased to 5 to 10 ml/min/100 ml. It appears likely that other peripheral circulatory beds such as the ear capillaries undergo similar changes and that the initial low volume flow will contribute to the prolongation of the time components in the first 24 hours of life and may account for the difference in AT MCT and time of onset of systemic resuscitation on the first day as compared to the following days, Fig 7

Summary

In 89 normal full term newborn infants from healthy mothers with uneventful pregnancies labors and deliveries, indicator dilution curves were recorded using indocyanine green and a Cambridge recording apparatus In 15 out of 17 infants studied curves were obtained during the first 24 hours of life which indicate the presence of a left to right shunt. Between days 2 and 8 twenty two out of fifty-four infants showed the left to right shunt pattern. Seventeen infants had a normal type curve and one had an undetermined type curve Fifteen showed mixed type curves during the same investigational period (normal, shunt or an undetermined type curve) After the 8th day only one shunt type curve was seen in 35 curves obtained from 18 infants.

In the newborn infants a prolongation of the time-concentration components was observed which gradually fell with increasing age of the infants The difference between these time-concentration components on the first day as opposed to the values obtained between the thirteenth and twenty third day is statistically significant.

The factors shortening the time-concentration components are discussed with an increasing flow velocity considered to be the most important

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From the Obstetric Medicine Research, University Aberdeen

Cord Around the Neck. Further Analysis of Incidence[1]

by J SELWYN CRAWFORD

Recently it was suggested [1] that amongst primigravid patients, the incidence of cord around the neck at delivery was one in three. The series, as published, consisted of 598 consecutive deliveries of married primigravidae with single pregnancies, who received their antenatal care under the supervision of the Obstetric Medicine Unit Aberdeen, and who gave birth to an infant of at least 2500 g birth weight Cases of breech presentation were not included in the study

The figures were based upon an analysis of data collected in large part by midwives and it was felt that they were reliable: the results of personal observation of a smaller number of cases had been in close agreement with those of the major series in approximately 90 % of cases of nuchal cord, the cord had been clamped and cut prior to completion of delivery and this positive involvement by the midwives appeared likely to diminish the possibility of recorder-errors.

However as cord around the neck is one of the factors which appears significantly to influence the findings obtained in a current investigation of the biochemistry of the newborn (result shortly to be published) it seemed appropriate to re-examine the data collected during the course of the previous study in a quest for maternal factors associated with nuchal cord The results of this further analysis are presented here

Consideration here is given to:

the original 598 patients;

eleven patients definable in terms similar to those enumerated in the opening paragraph, but delivered by Caesarean section;

thirty-six deliveries which conformed to the general classification except that the infant weighed less than 2500 g at birth (nuchal cord was present in 11 of these cases)

The possibilities that nuchal cord occurred more or less frequently in groups of mothers who had toxaemia of pregnancy or whose labour lasted for more than 18 hours, or who were aged 25 years or more were investigated As might be expected, no significant variation was found. The incidence of nuchal cord in a group of patients who had completed 4 weeks of pregnancy prior to delivery was no different from that of the entire series.

Attention was next turned to the possibility that there was an association be-

[1] Statistical consultation and computing services were provided by the Biological Sciences Computation Center University of Chicago (NIH Grant No. FR0013).

TABLE 1 *Examination of the entire series with reference to number of completed weeks of pregnancy*

Presented in the subsidiary Tables A, B, and C are data referred to in the discussion of the statistical analysis of the results (see text)

Completed weeks	<35	36	37	38	39	40	41	42	43	Total
With cord	5	3	6	11	37	78	52	20	3	215
Without cord	13	8	18	51	70	137	83	43	5	430
Total	18	11	24	6_	107	215	135	65	8	645
Cord incidence	0.28	0.27	0.25	0.18	0.35	0.36	0.39	0.31	0.38	
		0.217					0.358			0.333

Table 1A

Completed Weeks	<39	40	Total
With cord	62	78	192
Without cord	160	137	280
Total	222	15	57
Cord incidence	0.28	0.36	0.34

Table 1B

Completed Weeks	<38	39	Total
With cord	25	37	6
Without cord	90	70	160
Total	115	107	222
Cord incidence	0.22	0.35	0.28

Table 1C

Completed Weeks	<38	>39	Total
With cord	25	190	215
Without cord	90	340	430
Total	115	530	645
Cord incidence	0.22	0.36	0.33

tween gestational age and the incidence of nuchal cord

The hypothesis to be tested with regard to the data presented in Table 1 is that the incidence of cord is the same in all gestational age groups. Chi-square computed for Table 1 was 10 77 (8 deg fr) corresponding to a P value of 0.21 and not considered statistically significant For a more detailed analysis, thus X^2 was partitioned (cf 2) arbitrarily into 8 independent chi squares (1 degree of freedom each) corresponding to the 8 inquiries whether cord incidence in each age group (except the youngest) differed from cord incidence in the combined younger age groups. Two of these latter X^2's were moderately large for Table 1A, $X^2 = 3\ 43$ $P = 0\ 07$ for Table 1B $X^2 = 4\ 11$ $P = 0\ 05$. Thus there is some evidence albeit borderline in favour of differences in cord incidence among the gestational age groups

The data indeed show a considerable increase in cord incidence between the completion of 38 weeks and the completion of 39 weeks. If as suggested by this observation, Table 1 is compressed into Table 1C the corresponding $X^2 = 7.38$, which would be significant statistically ($P = 0\ 005$) had the decision to form Table 1C not depended on the data. These derivations thus give rise to an interesting hypothesis that could be tested specifically on a different set of data.

The relationship between gestational age and nuchal cord was maintained irrespective of the presence or absence of the factors in obstetric history mentioned previously

There remained one further possible explanation to be tested perhaps the infants of low gestational age being relatively smaller than the more mature infants, might slip through the loop of cord and

TABLE 2. *Consideration of entire series incidence of nuchal cord within groups defined with reference to birth weight*

Birth weight (g)	< 2000	2001–2500	2501–3000	3001–3500	3501–4000	4001–4500	> 4501	Total
With cord	5	8	48	92	53	7	2	215
Without cord	6	19	90	178	110	25	2	430
Total	11	27	138	270	163	32	4	645
Cord incidence	0.455	0.296	0.348	0.341	0.325	0.219	0.500	0.333
	0.343		0.343			0.312		
	0.347				0.328			

thus be free from nuchal cord at delivery. The series was, therefore, examined with reference to the birth weight of the infant (Table 2). There was no evidence that nuchal cord occurred less frequently among smaller infants than among larger.

The *major hypothesis derived from the* data obtained in Aberdeen was tested by applying it to a series which has been studied at the Chicago Lying In Hospital (Table 3). Virtual primigravidae (i.e. patients whose only previous pregnancy had resulted in an abortion during the first trimester) and primigravidae have been considered together and although the numbers are too small to provide statistical *significance they appear to strengthen* the hypothesis.

It is interesting to note that if there is indeed an increase in the incidence of nuchal cord among multigravidae as gestational age progresses, it occurs approxima

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TABLE 3. *Analysis of the incidence of cord around the neck with reference to gestational age at delivery in a small series of patients delivered at Chicago Lying-In Hospital*

Completed weeks	< 35	36	37	38	39	40	41	43	Total
Multigravidae									
With cord	3	2	0	10	13	9	0	1	38
Without cord	4	3	10	22	37	24	2	0	101
Total	7	4	10	32	40	33	2	1	139
Cord incidence			0.243				0.307		0.273
		0.236					0.290		
Primigravidae and virtual primae									
With cord		0	3	3	7	4	2	0	1
Without cord	2	4	7	11	10	14	2	2	5
Total	4	4	10	14	17	18	4		3
Cord incidence			0.25				0.317		0.298

tely one week earlier than is the case among the primigravidae Speculation re garding the cause of the distinction would be premature at this time

Discussion

As far as the writer is aware it has not previously been suggested that the incid ence of nuchal cord may be correlated with any maternal factor although Shui & Eastman [3] demonstrated that it was related to the length of the cord It ap pears from the present study that the in cidence is indeed not influenced by ma ternal age the duration of labour or the presence or absence of maternal hyperten sion or toxaemia However it is suggestive from the present analysis that the like lihood of finding a nuchal cord at the deli very of a primigravid patient (whose single foetus is presenting by the vertex) increases markedly when pregnancy ad vances beyond the 38th week. The expla nation of this increase should it be con firmed, might relate to such phenomena as lightening" increased activity of the foetus in utero and the diminution of the volume of amniotic fluid during late pregnancy A discussion of such hypo theses at this stage would probably be unrewarding It is however worth em phasizing that in any study—whether based upon clinical observation or upon a study of cord blood or intervillous blood—of the effects of increasing gestational age care must be taken that the influence (pre- or intradelivery) of nuchal cord does not distort estimations of the comparisons of the efficiency of exchange at the pla cental barrier

For example Suppose a number of primigravid patients, who were delivered during the 38th week of pregnancy and who were apparently free from obstetric pathology were investigated to provide base-line parameters of the biochemistry of intervillous blood or of cord blood, or of the incidence of neonatal depression; then suppose a similar investigation were undertaken upon a group of primigravidae similar in all other respects except that they were delivered at 40 weeks or 42 weeks gestation, if cases of nuchal cord were not excluded from the study there would possibly be twice as many such cases among the second group as among the first and thus the validity of many conclusions drawn from the results of the study would be open to grave doubt

Finally it must be emphasized that this study was concerned with primigravidae only No comparable data have yet been collected with respect to the multigravid patient

Summary

A re-examination of data referred to in previous communications has been made. The patients studied were all married primigravidae with single pregnancies presenting by the vertex, who had attended for antenatal care under the jurisdiction of the Obstetric Unit in Aberdeen. The series is comprised of 600 mature infants (including 11 delivered by elective Caesarean section) and 36 infants premature by birth weight.

Nuchal cord was found at delivery in one-third of these cases. Closer analysis revealed that the incidence was approximately the same among groups of patients who had one of the following attributes. hypertension or toxaemia of preg

nancy labour prolonged to or beyond 18 hours; aged 35 years or more at the time of delivery gestational period beyond the 42nd week.

Further analysis with reference to gestational age showed that the incidence of nuchal cord rose abruptly during the 39th week, and that the incidence thereafter was maintained during subsequent weeks.

It appeared that the birth weight of the infant was not a determining factor in this relationship.

The importance of this with respect especially to the investigation of the significance of increasing gestational age is briefly discussed.

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Acid Phosphatase Activity of Serum Thrombocytes and Erythrocytes in a Juvenile Form of Gaucher s Disease

by PER-OLOF HILLBORG and BENGT ESTBORN

The level of serum acid phosphatase is raised in Gaucher s disease (GD) [40 41 42] To elucidate the pathogenesis of this disorder of lipid metabolism it would be of great value if the origin of this phospha tase could be revealed. It is not known whether it is derived from a particular organ or from the whole reticuloendothelial system (RES) Neither is it known if the appearance of this phosphatase in the serum is the cause of the various symptoms of the disease or if it is a side effect of another enzyme error

In 1959 seven families from the Norr botten County of Sweden with 1_ cases of GD in children of a single generation were reported [23]. By examining the parish registers, it has been possible to show that 6 of these 7 familiesare related to each other andare descended from a common ancestor born about 1630 (Fig 1)

By investigating the 6 patients with GD and some of their relatives the au *thors have tried to find the answer to 4* different problems of importance in the disease

1 The symptoms of this Swedish form of GD are somewhat different from those of the classical adult and infantile forms. In this juvenile form neurological signs develop at a later stage than in the infantile form and the patients survive until adolescence [22]. Our aim has been to investigate whether the acid phosphatase level is also raised in this variant of the disease and whether the enzyme activity shows any characteristic deviations from the normal

2 Part of the acid phosphatase activity in serum is normally derived from the thrombocytes [44 49] The megakaryocytes also contain acid phosphatase [35]. There is therefore reason to investigate if the serum acid phosphatase in GD comes from the megakaryocytes-thrombocytes. We therefore examined the content and type of acid phosphatase in the thrombocytes in GD to find out if they deviate from the normal and if the type is the same as in diseased serum

3 It is known that normal erythrocytes contain a relatively large amount of acid phosphatase [28 36]. We have tried to clarify if the serum acid phosphatase in GD is derived from the erythrocytes, by analysis of the phosphatase content of the erythrocytes and by experiments with various enzyme inhibitors.

4 Gaucher s disease of this juvenile type

is inherited with an autosomal, recessive gene [35]. Since the parents and some of the siblings then are gene carriers it has been of interest to examine whether these relatives have a raised level of serum acid phosphatase.

Material

The patients are the same 6, described in a previous paper on the neurological signs in this form of GD [22]. In addition to the patients there is a group of relatives consisting of 3 grandparents, 11 parents and 9 siblings. A number of parents and siblings belong to families which have had children with GD now dead.

In 195., Emmrich & Scheffler showed that the serum level of acid phosphatase varies with age [9]. At the beginning of our investigation we knew of no work on the content of serum acid phosphatase in children, estimated by our method. In our studies, to be able to refer to the normal values at different ages, we first measured the phosphatase activity in groups of healthy children and adults.

We did not have access to a sufficiently large group of completely healthy children. We therefore contented ourselves, in the age group 4–20 years, with patients admitted to Kronprinsessan Loviesa Barnsjukhus for common paediatric conditions, not having metabolic or skeletal disorders, in which a raised or lowered acid phosphatase level presumably occurs [4, 8, 10, 15, 20, 21, 27, 29, 32, 42, 47]. Blood samples were taken from 58 of these children. In addition there were blood samples from 10 children admitted to the Stockholm City Hospital for Infectious Diseases with scarlet fever and 4 blood donors between 18 and 20 years of age. In this way the child-adolescent group in this "normal material" contained 72 persons.

The normal values for adults were determined in 52 blood donors between 21 and 60 years of age who had undergone a routine medical examination and been found healthy. The age distribution in the normal material is shown in Table 1.

Methods

Serum, citrated plasma rich or poor in thrombocytes and suspensions of thrombocytes and erythrocytes were collected from the normals and the patients and then prepared as described elsewhere [17]. For practical reasons only citrated plasma could be obtained from the patients' relatives. Because of primitive laboratory facilities, the samples from this last group could not be centrifuged. The red blood cells were allowed to sediment at +4°C for about 5 hours, and the plasma decanted off. The plasma samples were then kept deep frozen until analysis was possible.

The activity of the acid phosphatases was determined by the method of Stolbach *et al.* [38] with minor modifications as described elsewhere [12]. The substrat was 50mM disodiumphenylphosphat. The activity in serum and plasma was expressed in King-Armstrong Unit (KAU). One unit corresponds to the amount of acid phosphatase in 100 ml serum, which liberates 1.0 mg phenol from phenylphosphat at a pH of 4.9 at 37°C in one hour. The activity in thrombocytes and erythrocytes was expressed in μg phenol liberated at 37°C/hour/million cells.

The sensitivity of the acid phosphatases to inhibitory substances was examined using buffer solutions with 2mM copper sulphat.

TABLE 1. *Age and sex distribution of the normal controls*

Age	Males	Females	Total	
4–9 years	14	13	27	
10–15	17	21	38	2
16–20	7	0	7	
21–60	52	0	52	52
Total	90	34		1 4

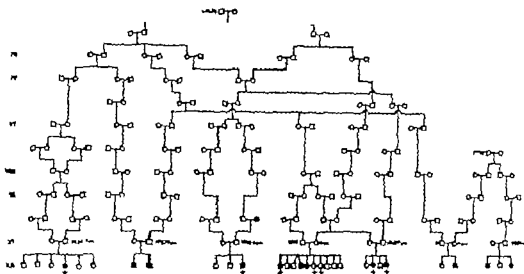


Fig. 1. Family tree of 7 families with Gaucher disease in the Norrbotten County of Sweden. Six of these families have a common ancestor born about 1630 in the community of Överkalix. ◻ = Healthy male. ● = Sick female.

0.5% formaldehyde and 20mM L(+)tartrate respectively.

Statistical calculations of the mean standard deviation and significance of the differences between the means (t test) were performed as described by Kemp & Nielsen (*8).

Results

As shown in Fig. 2 children and adolescents have a considerably higher acid phosphatase activity in serum than adults and the values have a relatively wide scatter. The activity then falls between the ages 12 and 20 years becoming rather constant in adults. Table 2 shows the material divided into various age groups. The mean for the child-adolescent group, 3.62 ± 0.94 (S.D.) KAU is significantly higher than the mean for the adults, 2.11 ± 0.48 KAU ($P < 0.001$).

As seen in Fig. 2 and Table 2 the acid phosphatase activity in serum from patients with GD is markedly increased. The differences are statistically highly significant if the patients' phosphatase values are compared with those of the normal controls in the corresponding age groups.

The acid phosphatase content of the thrombocytes and erythrocytes was estimated in 17 children and *0 adults from the normal material as well as in 6 patients. Table 3 shows that there is no significant

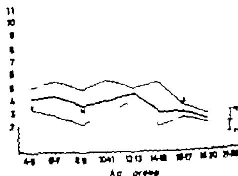


Fig. 2. Total acid phosphatase activity in serum from 6 patients with Gaucher's disease (●) and from normal controls in various age groups. For the ages 4–20 years the mean, maximum and minimum values are recorded, for the age group 21–40 years the mean ± S.D. is shown.

TABLE . Total acid phosphatase activity in serum from patients with Gaucher's disease and from normal controls

			Acid phosphatase activity K.A. Units	Statistical significance of difference between groups	
Gaucher disease					
A. Pat. J. Ö.	5 years		9.66	A–B	$P<0.001$
S. Ö.	7		8.64		
L. J.	9		11.41	BI–BII	$P>0.1$
J. J.	12		8.76	BI–BIII	$0.01>P>0.001$
A. A.	13		7.15	BII–BIII	$0.01>P>0.001$
K. J.	10		5.72		
		Mean ± S.D.	7.72±1.97	B–C	$P<0.001$
Normals					
B. I	4–9 years	$n=27$	2.85±0.80	BI–C	$P<0.001$
II	10–15	$n=38$	2.64±0.93	BII–C	$P<0.001$
III	16–20	$n=7$	2.66±1.10	BIII–C	$P>0.1$
B.	4–20	$n=72$	2.62±0.94		
C.	21–60	$n=82$	2.11±0.45		

difference between the acid phosphatase content of these cells in healthy children and adults. However the mean acid phosphatase activity of the patients' thrombocytes and erythrocytes is somewhat lower than that of the normal controls. Most of the individual values lie within the normal range (Fig. 3), but for the erythrocytes the difference between the means is highly significant ($P<0.001$).

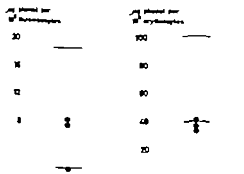


Fig. 3. Acid phosphatase activity in thrombocytes and erythrocytes from 6 patients with Gaucher' disease (●). The rectangles designate the normal range (±2 S.D.) determined on blood from 37 normal controls

The inhibitory effect of various substances on the acid phosphatase activity in serum, thrombocytes and erythrocytes was estimated in blood samples from the 6 patients and from normal children and adolescents between 4 and 20 years of age. As shown in Table 4 and Fig. 4, the inhibitory effect of copper sulphate and formaldehyde is lower in the serum and plasma of the patients than in those of the normals. The difference is highly significant. On the average L(+)-tartrate inhibits less than 5% of the acid phosphatase activity in serum and plasma of both the patients and controls. On the other hand the inhibitory patterns in thrombocytes and erythrocytes from both the patients and controls are identical (Table 4, Fig. 5).

To find out if the serum level of acid phosphatase is raised in the presumptive gene carriers we analysed blood samples from 3 grandparents, 11 parents and 9 siblings of the patients. Only plasma samples were taken from these relatives. Because of primitive laboratory facilities the

TABLE 3 *Acid phosphatase activity in thrombocytes and erythrocytes from patients with Gaucher's disease and from normal controls*

	Microgram phenol liberated/hour	
	10^9 thrombocytes	10^9 erythrocytes
A. Pat. J Ö 5 years	0.077	0.400
S. Ö 7	0.144	0.336
L. J 8	0.011	0.449
J. J 12	0.033	0.355
A. Å. 13	0.095	0.655
K. J 19	0.083	0.467
Mean ± s.d. $n = 6$	0.074 ± 0.047	0.446 ± 0.178
B. Normals 4–14 years $n = 17$	0.095 ± 0.039	0.720 ± 0.163
C. Normals 20–58 years $n = 20$	0.105 ± 0.049	0.734 ± 0.167
B + C. Normal 4–58 years $n = 37$	0.100 ± 0.044	0.722 ± 0.165
Difference B–C	$P > 0.1$	$P > 0.1$
A–(B + C)	$P > 0.1$	$P < 0.001$

samples could not be centrifuged satisfactorily so that many of the thrombocytes remained in the plasma. These samples were kept deep frozen. When the analyses were made later varying amounts of acid phosphatase had been released from the thrombocytes [49] so that the acid phosphatase level was raised.

Table 4 and Fig 4 and 5 show that the thrombocyte phosphatase is almost completely inhibited by copper sulphate while the plasma phosphatase in GD is very little inhibited We therefore examined the activity of copper resistant acid phosphatase of plasma from 69 of our normal controls, the patients relatives mentioned above and the patients. All of the relatives examined had values within the normal range but the patients had raised levels of acid phosphatase in plasma even after inhibition with copper sulphate Thus it is not possible to reveal the heterozygotes of the GD gene by phosphatase analyses Crocker & Landing obtained the same result [6]



Fig. 4 Inhibition patterns of acid phosphatase activity in serum (*S*) and plasma (*P*) from 1mM copper sulphate, 0.5 % formaldehyde and 20mM *L*(+)-tartrate – Normals, – Patients with Gaucher's disease.

TABLE 4 *Inhibition of acid phosphatase activity in serum plasma thrombocytes and erythrocytes from normal children (4–20 years) and patients with Gaucher's disease (5–19 years) by copper sulphate formaldehyde and* L(+)-*tartrate*

	Inhibition in per cent by					
	Copper sulphate		Formaldehyde		L(+)-tartrat	
	n	M ± S.D.	n	M ± S.D.	n	M
Serum						
A. Normals	50	11.1 ± 8.8	49	22.0 ± 7.8	50	< 5
B. GD	6	2.6 ± 2.6	6	11.0 ± 9.6	6	
Difference A–B		$P < 0.001$		$P < 0.001$		
Plasma						
C. Normals	39	18.8 ± 9.4	31	29.8 ± 11.1	31	< 5
D. GD	6	4.9 ± 5.1	6	14.8 ± 4.6	6	
Difference C–D		$0.01 > P > 0.001$		$P < 0.001$		
Thrombocytes						
E. Normals	9	92.4 ± 2.8	9	98.5 ± 2.2	9	< 5
F. GD	6	90.5 ± 2.1	6	97.2 ± 1.5	6	
Difference E–F		$P > 0.1$		$P > 0.1$		
Erythrocytes						
G. Normals	9	93.2 ± 2.7	9	93.0 ± 2.8	9	< 5
H. GD	6	94.7 ± 1.7	6	97.5 ± 1.7	6	
Difference G–H		$P > 0.1$		$P > 0.1$		

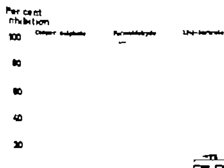


FIG. 5. Inhibition patterns of acid phosphatase activity in thrombocytes (*T*) and erythrocytes (*E*) from 2mM copper sulphate, 0.5 formaldehyde and 20mM L(+)-tartrat — Normals, — Patients with Gaucher's disease

Discussion

In their very thorough work on serum acid phosphatase in GD Crocker & Landing also gave normal values for children [6] They used phenylphosphate as substrate. Their normal controls were 39 institutionalized children from a state hospital, free from bone or metabolic disease and 41 healthy schoolboys. They found a clear difference between the levels of acid phosphatase in serum of children and adults.

In their investigation Laron & Epstein-Halberstadt [31] had a normal material of 101 healthy children from newborn to 18 years of age as well as 13 adults. This is presumably the largest and most repre-

utative investigation in this field in the literature The highest level was found during the first two days of life

In the above two papers there was a rise in serum acid phosphatase at 12–13 years of age and the level in children was about twice as high as in adults. These results were confirmed by us. In our material the difference between the combined child-adolescent and adult groups is highly significant (P 0 001)

Gebala & Rosenmund could not find any difference between the activity of serum acid phosphatase in 30 children 1–1 year of age and in adults [16]. The explanation may be that they used β-glycerophosphate as substrate

Serum acid phosphatase activity in Gaucher's disease

Apart from metastasising prostatic cancer and GD high serum acid phosphatase has been found in Paget's disease [20 47] hyperparathyroidism [29] osteopetrosis [10 43] osteogenesis imperfecta [15 27] liver disease [4] gonadal dysgenesis [8] mammary cancer [32] and Niemann Pick's disease (NPD) [5 21] Except for the last disease the clinical differential diagnosis from GD should present no great difficulties It is interesting that in 11 determinations on 6 different patients with NPD Crocker & Farber [5] were unable to confirm the rise of acid phosphatase described by Hastrup & Videbaek [21] in a patient with this disease However the acid phosphatase in this last patient was of the type found in GD with low activity to β-glycerophosphate and high to phenylphosphate

Rozenszajn & Efrati [37 personal comm.] have investigated the staining properties and appearance of the GD cells and the level of serum acid phosphatase in 6 patients. Four of these patients had GD cells in which the cytoplasm gave typical staining reactions and contained curved fibrils often arranged in whorls. The serum acid phosphatase level was raised in these 4 patients. The fifth patient had typical GD cells in the bone marrow but normal value of serum acid phosphatase and a nonpalpable spleen. The GD cells of the sixth patient showed similar staining reactions but the cytoplasm was filled with vacuoles and thus resembled NPD cells. This patient's spleen was enlarged and the serum acid phosphatase was raised

If the diagnoses were right the case of NPD of Hastrup & Videbaek and the sixth case of GD of Rozenszajn & Efrati suggest that there may be intermediary forms of these diseases, or that neither the appearance of the lipidosis cells nor the serum acid phosphatase level is completely reliable in the differential diagnosis of these two diseases. Chemical analysis of spleen or liver lipid content may be necessary in addition.

Acid phosphatase activity of thrombocytes and erythrocytes

The content of p-nitro-phenyl-phosphatase in erythrocytes in different types of anaemia has recently been studied by Valentine *et al* [45] and Vaspio [45]. They observed subnormal values only in occasional cases of thyrotoxicosis, leukaemia and chronic renal failure The significance of the lowered erythrocyte acid phosphatase activity in the present series is not clear However this latter should be investigated in detail, since it implies that other metabolic disturbances may occur

in GD erythrocytes It may thus be possible to find a way of revealing heterozygotes.

Inhibition patterns

In their investigation of serum acid phosphatase activity in 12 GD patients, Tuchman *et al* [4] using the 3 substances mentioned above found greater inhibition than we From their tables it is possible to calculate the inhibition to an average of 9 6% with copper sulphate 26 6% with formaldehyde and 11 4% with tartrate. However the age distribution in the 2 series was different Nine of their 12 patients were over 20 years of age while all of ours were less than 20 The authors did not show any normal inhibition patterns.

Crocker & Landing [6] also found greater inhibition of GD serum acid phosphatase than we viz. 30% with formaldehyde and 18 with L-tartrate but they made no comparison with normal inhibition

The subnormal inhibition of serum acid phosphatase activity by copper sulphate and formaldehyde in our GD patients seems to imply that the increased activity depends largely on an enzyme which normally contributes very little to the total serum acid phosphatase activity

Thrombocyte and erythrocyte acid phosphatases are both inhibited 92–98% by copper sulphate and formaldehyde but less than 5% by L(+)-tartrate thu agreeing with the erythrocyte phosphatase results of Abul Fadl & King [1] and the thrombocyte phosphatase results of Zucker & Borelli [49]

Acid phosphatase derived from thrombocytes and erythrocytes is very strongly inhibited by copper sulphate and formaldehyde The slight and subnormal inhibition of the increased acid phosphatase activity in GD serum by the same substances is very much against the origin of the enzyme from these cells.

Abul Fadl & King have shown that the acid phosphatase from prostate liver spleen and kidney are strongly inhibited by L-tartrate [1] Since GD serum acid phosphatase is but little inhibited by this substance Tuchman *et al.* considered that it could not be derived from these organs [43]

Abul Fadl & King found that liver cid phosphatase was 70% inhibited by L-tartrate while the acid phosphatase of bile was unaffected, presumably due to a bile inhibitor of L tartrate inhibition. If GD serum acid phosphatase were formed in the thrombocytes or erythrocytes it should be strongly inhibited by copper sulphate and formaldehyde but this is not so (Fig 4 and 5) The latter could be explained as inhibition of these two inhibitors by a factor in GD serum by analogy with Abul Fadl experiment We have excluded this possibility [11]

Th orign of the increased serum acid phosphatase act vity in Gauche s disease

Our investigation has not elucidated the problem of where the GD phosphatase is formed nor whether the presence of this phosphatase is the cause of or a ide effect of the disease Crocker & Landing [6] observed that the level of serum acid phosphatase in GD decreased after splenectomy but later slowly increased. We were ble to confirm this in a 4-year-old girl with juvenile GD not included in this series. Both Crocker & Landing and

Rozenszajn & Efrati [37] showed that GD cells contain large quantities of acid phosphatase The above findings strongly suggest that GD phosphatase is formed in the GD cells and leaks out into the serum Splenectomy removes the largest depot of GD cells so that the serum acid phosphatase level falls. When GD cells in other parts of the body have formed sufficiently large quantities of phosphatase the serum level rises This is the most plausible explanation of the origin of the GD phosphatase

Meijer & Willighagen have shown [33] that after intraperitoneal injection in mice macromolecular substances are stored in RE cells in spleen and liver with increase in their acid phosphatase activity and enlargement of the organs. Becker & Barron [3] reported that after anoxic-ischaemic damage of the central nervous system (CNS) in rats, swelling of nerve cell lysosomes, containing acid phosphatase occurred at an early stage but that in older infarcts *the* picture was that *of loss* of acid phosphatase activity In their investigations on human pathologic tissues from the CNS Anderson & Song [2] found intense acid phosphatase reactions in adventitious cells in hyperplastic blood vessels and in macrophages close to tumour necrosis.

These recent results may explain why *the serum acid phosphatase increases so* strongly in GD The authors suggest the following hypothesis

Gaucher's disease depends on a metabolic defect the fundamental nature of which is unknown. An increased quantity of glucocerebrosides circulates in the blood At present it is not known whether creased breakdown of normal cerebrosides *or whether cerebrosides of abnormal structure are formed.* The circulating glucocerebrosides are phagocytosed by RE cells The process of phagocytosis causes a great increase of acid phosphatase production in the phagocytes When these become necrotic mostly in the spleen, the acid phosphatase is released into the plasma

The cells of the RES also proliferate in the adventitia of the brain vessels [7]. In some places this proliferation goes so far as to form cuffs of GD cells round small and medium-sized vessels constricting them and producing ischaemia. The patients with infantile or juvenile GD *often* have lung changes with a poor oxygenation of the blood resulting in anoxic-ischaemic damage with small necrotic areas in several organs and increased phagocytosis. In this way the level of acid phosphatase in serum is raised still more

If this hypothesis is correct, it implies *that* the occurrence of the GD phosphatase is a secondary phenomenon and of no primary pathogenic significance in the disease. However the effect of phagocytosis may not be the whole explanation of the presence of the GD phosphatase and the authors have started new experiments with the following motivation

In human organs cerebrosides normally *occur in relatively large quantities only in* the CNS so that the cause of a disturbed cerebroside metabolism should first be looked for here Wolf *et al* [46], Ghi & Datta [17], Fleischhacker [14] and Anderson & Song [2] have investigated the acid phosphatase content of the CNS. According to Wolf the nervous system is one of the most consistent sites of acid phospha

tase activity Anderson & Song found that neurons and choroid plexus epithelium have the strongest activity of acid phosphatase in the CNS

Investigations of the properties of acid phosphatase in nerve cells and glia cells from various parts of the CNS may reveal the primary metabolic defect in GD The authors have started such an investigation in normal brain for later comparison with GD brains.

Summary

The serum level of acid phosphatase is raised in Gaucher's disease (GD) In Sweden a juvenile form of the disease with neurological signs, is found. The authors have shown that the serum acid phosphatase level is also raised in this variant

Gaucher's disease is inherited with an autosomal, recessive gene Both the parents and the siblings of the patients had normal acid phosphatase values Thus heterozygocity for the gene cannot be demonstrated by phosphatase analyses.

The patients erythrocytes contained significantly less acid phosphatase than normal. Continued investigations will show if this can reveal the heterozygotes.

Copper sulphate and formaldehyde only inhibited the serum acid phosphatase a little in GD and less than normally The acid phosphatase of tes and erythrocytes was inhib com pletely by the same inhil t he in hibition was the same for I nts and normals This shows that m acid phosphatase in GD is of a er than the normal serum acid pl and that it is not formed in throm or erythrocytes.

During the last few years vari vestigators have shown that phagoc form large quantities of acid phosphata during the phagocytosis of macromol cular substances To explain the raised level of serum acid phosphatase in GD the authors submit a theory based on the new findings

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Clinical Evaluation of an Oral Anabolic Steroid (Methandrostenolone, Dianabol CIBA) in Children with Muscular Weakness and Wasting

by INGRID GAMSTORP

The diseases of infancy and childhood leading to progressive muscular weakness and wasting constitute an important pediatric problem. New methods have increased the possibilities of establishing a correct diagnosis and thereby of predicting the development of the disease and of calculating the risk of children in the family being affected, no form of treatment has, however proved successful. The present trial was prompted by encouraging reports about the effect of anabolic steroids in conditions characterized by muscular wasting and weakness [? 3 5 6 10 11 14 15 16]

Material and Methods

The material consisted of 21 children below 17 years of age who were admitted to the Department of Pediatrics in Lund between July 1 1961 and August 1 1963 because of muscular wasting and weakness. By now the patients have thus been followed between 7 months and 2½ years. Besides inquiry into the personal and family history and a careful physical examination, including neurological examination, investigation of the patients included electromyography measurement of conduction velocity of peripheral nerves, histological examination of a muscle biopsy specimen and determination of the activity in the serum of transaminase aldolase and lactodehydrogenase. A diagnosis, as specific as possible was based on the results of these examinations. The distribution of the material according to age sex and diagnosis is apparent from Table I

During the first few weeks the patients received only physical therapy in the form of active muscular training, care being taken to avoid overtraining and muscle trauma. Several of the patients improved during this treatment, which has then been continued with short interruptions regardless of other therapy given. Before treatment with anabolic steroids was started moving pictures were taken, the strength of different muscle groups was estimated, and the patient's capacity to move was also described verbally. It was noted whether the patient could perform certain defined movements, such as lifting his head when he was supine sitting up with or without help of his hands, with or without first rolling over in the prone position, getting up from sitting on the floor with or without climbing up on himself walking unassisted, climbing stairs with or without help of his hands.

Treatment with an anabolic steroid was

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TABLE I *Distribution of the material according to age, sex and diagnosis*

Age in years	0–2		3–5		6–10		10–16		Total	
Sex	M	F	M	F	M	F	M	F	M	F
Spinal muscular atrophy										
Polio				1						1
Progressive infantile type (Werdnig Hoffmann)	2									
Progressive, juvenile type (Kugelberg Welander)				2		1				3
Amyotrophic lateral sclerosis							1		1	
Hereditary chronic polyneuropathy	1?		1?					1	?	1
Primary muscle disease										
Progressive muscular dystrophy (type Duchenne or limb-girdle)					6		2		8	
Undetermined type	1				2				3	
Total	4	0	1	3	8	1	3	1	16	5

then started. Methandrostenolone (Dianabol CIBA) was chosen because it is given orally which is a great advantage in the treatment of children. The dose was 0 1 mg per kg body weight per day given for periods of 3 months with free interval of 4–6 weeks. In some patient the dose was doubled during subsequent courses. The number of courses given depended on the effect and side-effects.

During the entire period of intermittent treatment the patients were re-examined clinically at intervals of 1½–3 months. Changes in the children behaviour observed by the parents were recorded, as was the patient subjective estimation of the effect of the treatment A note was made of any improvement or deterioration of specific functions as previously described New moving pictures were taken once every 3–4 months. Side-effect were also apparent from the clinical examination, repeated measurement of the height and weight of the child, estimation of the skeletal age on X-ray films, the urinary excretion of 17 ketosteroids, and liver function tests.

Results

The results are summarized in Tables 2 and 3

Three patients could only be followed up for 3–6 months, one had myopathy of undetermined type one had juvenile spinal atrophy and the third probably had polio-sequelae. During the same period one infant with the infantile type of spinal atrophy died from his disease the diagnosis was confirmed at autopsy One boy with progressive muscular dystrophy was lost to follow-up after an observation period of 1½ year the remaining 16 patients are still under observation.

In the group with muscular atrophy due to myelopathy one girl with probable *polio-sequelae* showed some improvement. She was a refugee originally believed to have a progressive disease However re-inquiry into her history suggested severe sequelae after polio or possibly RSSE (Russian spring summer encephalitis) as the probable cause of her symptoms. Her improvement was not greater than could be expected in a case of a non progressive disease The mothers of the boys with the *infantile type of progressive spinal atrophy*

TABLE 2 *Effect and side-effects in different diagnostic groups*

Diagnosis	No. of patients included in study	No of patients showing: No effect	Slight improvement	Moderate improvement	Improvement followed by rapid deterioration	Side-effects severe enough to cause discontinuation of treatment
Spinal muscular atrophy						
Polio	1		1			
Progressive, infantile type (Werdnig Hoffmann)	2	2				
Progressive juvenile type (Kugelberg Welander)	3	1				1
Amyotrophic lateral sclerosis	1	1				
Hereditary chronic polyneuropathy	3	1	1	1		
Primary muscle disease						
Progressive muscular dystrophy (type Duchenne or limb-girdle)	8	1	1		6	5
Undetermined type	3	1		2		
Total	21	7	5	3	6	6

claimed that the infants became stronger during treatment an impression which, however could not be confirmed on objective examination. One of the infants died from his disease during the second course of treatment The other boy has now been receiving intermittent treatment for 2 years Although no improvement has been noted, the disease has apparently not progressed during these 2 years. Of 3 girls with the *juvenile type of progressive spinal atrophy* one showed a steady progress of the disease apparently uninfluenced by the treatment One girl learned to get up from sitting on the floor and to climb stairs without help of her hands, unfortunately she was one of those lost to the follow-up (lost after 5 months) The third girl showed some improvement, she learned, e.g to rise from sitting on the floor During the following year no progress of her disease was observed. In the boy with *juvenile amyotrophic lateral sclerosis* the disease progressed steadily uninfluenced by the therapy

Of 2 brothers with a provisional diagnosis of *chronic hereditary polyneuropathy* one showed definite improvement, the other no apparent change during an observation period of 9 months. In a third patient in this group the diagnosis was firm and the disease unusually severe affecting both distal and proximal muscle groups Before treatment was started, the girl could not get up from sitting on a chair nor could she walk without support. She gained both these functions during her first course of therapy Improvement afterwards continued at a slower rate.

Two of the 8 boys with *progressive muscular dystrophy* were confined to wheel chair at the beginning of the treatment on examination no influence of the treatment was noted. However the parents of one

TABLE 3 *General survey of material and results*

Age in years at start of treatment	Sex	Diagnosis	Dose/kg and day	Duration of therapy	Effect	Side effects
4	F	Polio	0.1 mg	3 months	Less tired when sitting, could use her hands better for playing	Growth of pubic hair
11	M	Progr. inf. spin. musc. atrophy	0.1 mg	3 months	No definite change. Died 1 year later. Autopsy confirmed the diagnosis	None
1	M		0.1 mg	2 years	No definite change	None
3½	F		0.1 mg	1 year	Learned to sit up from supine position, to get up from sitting on floor	Pubic hair, phallic enlargement, deeper voice, acne, rapid increase of skeletal age
4	F	Progr. juv. spin. musc. atrophy	0.1 mg	3 months	Learned to get up from sitting on floor without climbing, learned to climb stairs without help of her hands	Slight growth of pubic hair. Lost to follow up shortly after first course
9½	F		0.1 mg	8 months	Progress of disease apparently uninfluenced by therapy	Pubic hair, acne, hoarseness
17	M	ALS	0.2 mg	2 years		None
[illegible]	M		0.1 mg	3 months	Learned to sit without support and to stand with support	None
5½	M	Chron. hered. polyneuropathy	0.15 mg	6 months	No definite change	None
14½	F		0.1 mg	1½ year	Learned to walk unassisted and to get up from sitting on a chair	None
15	M		0.15 mg	1½ year	No change on examination. Pt. feels stronger and better	None
15½	M		0.1 mg	1½ years	Learned to sit up without help of hands, walks better	None
8	M		0.1 mg	9 months	Learned to get up from sitting on floor without help of his hands, walked better; after 6 months deterioration again	Weight increase 9 kg, tough thickening of skin and subcutis, phallic enlargement
6½	M		0.1 mg	11 months	Improvement first 6 months, then deterioration	Tough thickening of subcutis and skin, increase in length and weight, phallic enlargement
8½	M	Progr. musc. dystrophy	0.1 mg	6 months	No definite change	Pubic hair, phallic enlargement, tough thickening of skin and subcutis, hoarse voice

Table 3 (continued)

Age in years at start of treatment	Sex	Diagnosis	Dose/kg and day	Duration of therapy*	Effect	Side-effects
8	M		0.05–0.1 mg	15 months	Improvement first 8 months, then deterioration	Pubic hair, phallic enlargement, tough thickening of skin and subcutis, hoarse voice
8	M		0.1 mg	3 months	Improvement first 2 months, then deterioration	Pubic hair, phallic enlargement, tough thickening of skin and subcutis
7	M		0.1 mg	1 year	Improvement first 3 months, then deterioration	
6½	M	Prim. muscle dis. of undet. type	0.1 mg	1 year	Learned to climb stairs without help of hands, walks better	Pubic hair, phallic enlargement
7½	M		0.1 mg	3 months	Learned to walk with braces but unassisted, lost to follow-up after 3 months	
½	M		0.1 mg	4 months	No definite change	

* Treatment was given intermittently in those cases where the duration of therapy exceeded 3 months.

of them believed that the boy had improved considerably and insisted on further intermittent therapy. As this severely handicapped boy may possibly feel better during such treatment and as he has shown no side-effects, their wish has been fulfilled. Of 6 boys ambulant at the beginning of the treatment, 5 were below 8 years of age; these 5 responded in largely the same way. During the first course they improved, learned to climb stairs, to get up from sitting on the floor without climbing on themselves etc. Then improvement stopped and the next course was of little value. During or after the second course rapid deterioration started and continued uninfluenced of all attempts to further treatment. One year after treatment had been started their disease had progressed to a stage that might have been expected if they had never been treated and never had this short period of improvement. Finally, the 15-year-old boy who at the beginning of the treatment was still able to walk without support, improved considerably during the first [illegible] courses of treatment and could then sit up from the supine position without using his hands, for example. Intermittent therapy has been continued for 2½ years. A slow progress has in the meantime occurred but he is still better than he was 2½ years ago.

Two of the 3 boys with *myopathy of undetermined type* had a stationary or very slowly progressive disease. Both improved considerably during treatment and one learned how to walk. The follow up period is, however, 3 months in one of

them and a year in the other The third boy probably has an infantile type of progressive muscular dystrophy apparently refractory to the treatment

Side-effects Some androgenic effects were seen, e.g phallic enlargement and growth of pubic hair in all girls and prepuberal boys. Although this was annoying to some of the parents, it never alone prompted discontinuation of the treatment. The appearance of acne and a coarse voice which occurred both in boys and girls, was disturbing to some of the patients. Of greater importance in these weak patients was the accelerated increase in height and weight noted in 6 of the prepuberal children. Skeletal maturation out of proportion to the child age and height was noted in one patient, in whom it was so severe as to contraindicate continuation of therapy closing of the epiphyses was, however not noted. A troublesome side-effect was a tough thickening of the skin and the subcutaneous tissue stiffening the children s joints and reducing their capacity to move about particularly in the young boys with muscular dystrophy No abnormal liver function tests were noted. Changes in the urinary excretion of 17 ketosteroid were insignificant

Discussion

The numerous attempts to treat conditions characterized by progressive muscular wasting and weakness reflect the changing concept of these diseases [0] As nothing known of the nature of a postulated specific defect in these conditions, it appeared reasonable to try a general protein-saving muscle-building substance Testosterone is such a substance but its androgenic side-effects are too strong to permit it use in females and prepuberal boys The introduction of substances with a high anabolic and a low androgenic effect has reduced this disadvantage Several groups of patients with muscular dystrophy have been reported to improve during treatment with anabolic steroids or at least to show an arrest of the previously steady progress [3 5 6 10 11 14]. Some authors have stressed that children with the Duchenne type of muscular dystrophy responded least to the therapy [_, 3 6] In one reported series no response to anabolic steroids was found in patients with neurogenic atrophy [11], in others a good effect of the same treatment has been described [10 15] Sture [15] report is of particular interest in this connection, because his material consisted only of children, in whom he found good results of therapy with anabolic steroids in 5 of 11 patients with progressive infantile spinal muscular atrophy His report prompted the inclusion in the present material also of cases of neurogenic muscular atrophy

However unfavorable results of treatment with anabolic steroids have also been reported. Dowben & Perlstein [7] emphasized the rapid deterioration, particularly in boys with the Duchenne type of muscular dystrophy after the cessation of treatment Fröhlich, Mumenthaler & Wiesendanger [8] found no effect of the treatment in their material which included one child and 22 adults with muscular dystrophy or lower motor neuron diseases. The absence of effect was also noted by Gyulai & Oreil [0] in one case of infantile spinal muscular atrophy and by Lundberg & Blom [13]; their material co

sisted of one child and 13 adults all with muscular dystrophy Barwick, Newell & Walton [1] reported the results of a double blind trial using methandrostenolone or nandrolone decanoate for periods of 3 months alternating with placebos for 3 months. No significant increase in muscular power or in functional capacity was noted in patients receiving the active remedies

The conclusion by Barwick *et al* [1] that anabolic steroids have no place in the routine treatment of cases of muscular dystrophy is valid also for the present material (here naturally confined to cases occurring in childhood) The short period of improvement noted in the patients with the Duchenne type of muscular dystrophy is of no value since it is followed by a rapid deterioration. The inspiration of vain hopes adds to the burden of the patient s parents and should be avoided.

None of the patients with spinal muscular atrophy showed such an excellent response as *that described by Stur [15].* [illegible] this connection the importance of a correct diagnosis has to be stressed. In Stur s cases the diagnosis was founded on clinical (weakness, hypotonia, areflexia) and electromyographic grounds However the clinical picture described may also be consistent with a diagnosis of chronic polyneuropathy and the needle electromyogram *does not always allow a distinc*tion between the 2 main causes of denervation i e myelopathy and peripheral neuropathy [12]. As pointed out by Byers & Taft [4] chronic polyneuropathy occurs in infancy and childhood, and it may be difficult to distinguish from the progressive myelopathy the prognosis of which is much worse It thus appears conceivable that Stur s [15] improved patients might *represent examples of chronic polyneuro*pathy in infancy In the present material unequivocal improvement was noted in the single case of hereditary chronic polyneuropathy

Some improvement was also noted in * girls with the juvenile type of spinal atrophy in one boy with probably chronic polyneuropathy and in 2 boys with a primary muscle disease of undetermined type The results are however hard to evaluate in these patients, as their diseases are slowly progressive or may even be stationary the difficulties are increased by the fact that no exact diagnosis could be established in some of the cases.

Virilization was, with the dose used, never severe enough to cause interruption of the treatment More serious side-effects were a too rapid increase of height, weight and skeletal age and particularly a tough thickening of the skin and the subcutaneous tissue which was noted mainly *in boys with progressive muscular dy*strophy in whom it counteracted the otherwise beneficial effect of physical therapy Impaired liver function, described during treatment with oral anabolic steroids [17 18], was not seen in the present material

Summary and Conclusions

An *oral* anabolic steroid, methandrostenolone (Dianabol CIBA) was tried on 21 children with muscular weakness and wasting due to a lower motor neuron disease or a primary muscle disease. The dose was 0 1–0.2 mg per kg body weight per day for 3 months with therapy free intervals of 4–6 weeks. The observation period was ½– ½ years

1 This therapy has no place in the routine treatment of progressive muscular dystrophy in childhood. The usual response noted in young boys with the Duchenne type of the disease was a moderate improvement, followed by a rapid deterioration.

2 The despairing course described in the previous paragraph was not seen in cases of neurogenic atrophy. Provided the disease is severe and no side-effects are noted, it therefore appears justified to try the drug in such patients. No case of myelopathy responded strikingly to methandrostenolone. Considerable improvement was noted in one case of chronic hereditary polyneuropathy.

3 A rapid increase of height, weight or skeletal age, noted in about a third of the prepuberal children, was considered an important side-effect. The most serious side-effect was a tough thickening of the skin and the subcutaneous tissue making the joints stiff and severely impairing physical therapy.

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CASE REPORT

Intestinal Function after Massive Resection of the Small Intestine in a Newborn

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Studies on patients following massive resection of the small intestine offer valuable information as to the site of absorption of different types of foodstuffs.

The literature dealing with extensive resection of the small intestine is voluminous. Havmond [3] collected 257 cases from the literature and added three cases of his own. The term *massive resection* has been used if 200 cm or more of the small intestine is removed. This length has been estimated to constitute about one-third of the small intestine in adults. The age of the patient was mentioned in 214 of the above cases, the youngest one being an 8-year-old boy. There are very few reports in the literature on the result of massive resection of the small intestine in small children. Swain *et al* [8] reported the course of 18 newborn infants following the resection of 5–67 cm of the small intestine. Seven of the infants died. The average length of the small intestine in newborn is, according to Potts [7] 308 cm and 67 cm is less than one-fourth of the small intestine in a newborn. There are at least three reports of cases of subtotal resection of the small intestine in infants.

Pilling & Cresson [6] observed survival of two newborn infants following resection of all but 28 cm of the small intestine including the ileocoecal valve. Clark & Booth [1] reported the result of the resection of about four fifths of the distal small intestine with jejuno-colostomy in a small infant. Wilkinson in July 1963 published the effects of removal of large part of the small intestine in 6 newborn infants and in one infant aged 6 months [9]. Five of the newborn infants died within 50 days while survival of the other two infants was recorded. All jejunum was left in the infant aged 6 months, and 60 cm of the jejunum plus 10 cm of terminal ileum in the surviving newborn infant.

This article deals with a case of massive resection of the small intestine in a newborn infant and follow up studies.

Case Report

B. S. M. is a female child born on February 16th, 1961. She was the product of an uneventful pregnancy and uncomplicated delivery at term (birth weight 3700 g and length 50 cm). She was admitted to the Central Hospital in Trondheim at the age of

4 day because of persistent vomiting since birth. At operation on the twelfth day volvulus and malrotation of the intestine were found. A week later relaparotomy had to be performed for ileus. Almost all the small intestine was found to be necrotic, and all but 20 cm of the jejunum and 10 cm of the distal ileum were resected. The post-operative course this time, was uncomplicated.

This child has since been admitted three times to the Pediatric Department of the Rikshospital.

She was first admitted at the age of 16 weeks with a diagnosis of acute gastroenteritis. A slow but steady weight gain had been maintained since discharge from hospital with an increase from 3070 g to 4330 g eight days prior to admission. The mother had nursed the infant 5 times a day and fed her milk formula twice. The infant had been passing 2–4 stools daily. Vomiting and diarrhoea started suddenly a few days prior to admission. There had been several cases of acute gastroenteritis in the neighbourhood. Physical examination on admission revealed a well developed 16-week-old infant. She was pale and mildly dehydrated, but she recovered quickly from her gastroenteritis. The concentration of haemoglobin was 12.9 – 12.8 g/100 ml, serum iron 68 microg/100 ml, and serum protein 5.8 g/100 ml, with decreased albumin fraction. Total lipids were 590 mg and serum cholesterol 108 mg/100 ml. The serum concentrations of calcium, phosphorus, chlorides and potassium were normal, while sodium was decreased to 128 mg/l. Her glucose tolerance test was flat. The stools are described as bulky but the total daily fat excretion was only 1.3 g. Plasma prothrombin was 30–44 % of normal, and increased to 64 % following an injection of 1 mg of vitamin K. The infant recovered rapidly during the hospital stay and she was discharged on the same type of feeding, with the addition of homogenized baby foods.

She was readmitted at the age of 7½ months and had maintained a steady weight gain during her stay at home. Supplemental iron had been started. Her weight had increased to 6180 g, and her length was 66 cm. Haemoglobin concentration was 14.3 g/100 ml, serum protein 6.3 g/100 ml with slightly increased albumin fraction. Plasma prothrombin was 19 %, and increased to 90 % following an injection of vitamin K. The stools were bulky, total daily fat excretion had increased to 11.. g, which was 65 % of faecal dry weight. The hospital stay was uneventful, and she was discharged with prescriptions for monthly B_{12} and vitamin K injections. (No B_{12} absorption studies were performed.)

The last admission took place at the age of 1 year and 9 months. She had been doing fine on restricted fat intake except for 2–3 periods of diarrhoea, probably precipitated by large food intakes. She walked alone at the age of 18 months. The mother had discontinued iron some time prior to the last admission because of loose stools. Vitamin B and K injections had been given regularly. Physical examination revealed a well nourished, well developed female infant, but she was short. Her height of 76 cm placed her 1 cm below 2½ percentile for her age, while her weight of 10.88 kg was at the 75 percentile for her length.

Laboratory studies at the age of 1 year and 8–9 months revealed that her haemoglobin concentration had remained high, 13.4 g/100 ml, haematocrit was 39 vol. %, serum protein 6.4 g, with albumin 4.0 g/100 ml. The serum electrolytes were normal, magnesium 1.9 mEq/l, total lipids 635 and cholesterol 120 mg/100 ml. Plasma prothrombin remained normal in spite of no vitamin K injections during a two months period. Glucose tolerance test showed an increase from 105 to 138 mg/100 ml.

During the first part of this hospital stay she passed 2–3 green, loose stools daily, but they changed to more normal colour and consistency when fat and roughage intake were restricted. The total amount of stools during three days period was 160–280 g, total faecal fat excretion 5.7 – 4.2 g. Xylose absorption was considered to be normal. Serum B_{12} was determined at the St. Joseph Hospital in Porsgrunn and found to be 277 picogram per 1 ml serum (two months after

the last B_1 injection). The absorption of radioactive vitamin B_{12} was measured by the method of Heinle *et al.* [4]. Using a test dose of 1 µg Co^{60} labeled vitamin B_{12}, a faecal excretion of 60% of the testdose was found. The absorption of vitamin B_{12} was thus normal, judged by absorption of radio labeled vitamin B_{12}.

Roentgenographic studies of the upper gastrointestinal tract were performed on all three admissions. The barium reached the coecum within 45 minutes the first time. The passage was considerably slower the second and third time, but still rapid. The small intestine remained very small, as shown on the figure. The total length of small intestine was estimated to be 50–60 cm. Gall bladder stones were seen this time.

Bone age was normal at the age of 10 weeks, but retarded at the age of 1 year and 8 months. General osteoporosis was also present.

In October 1963 we received a report from the child's physician. The child had developed remarkably well during the last year and could tolerate more fat in her diet. Her height at the age of $2\frac{3}{12}$ years was 87 cm (an increase of 11 cm during one year) and her weight 12.5 kg. Her haemoglobin concentration had remained high without any iron or B supplementations. Roentgenographic studies performed in September 1963 were interpreted as showing normal bone age.



Fig. 1. Roentgenogram of gastrointestinal tract of B.S.M. at the age of 1 $\frac{8}{12}$ years. The total length of the small intestine is estimated to be 50–60 cm. The coecum is located in the left side of the abdomen. The mucosal pattern of the small intestine is normal, but the distal part of the small intestine is slightly widened. Gall-bladder stones are seen.

Comments

The major problem following surgical removal of large amounts of the small intestine is maintenance of nutrition. The clinical picture will depend on the extent of intestine removed, and which part of the small intestine is left. Pathological changes in the remaining part of the small intestine are also of great importance. Surgical removal of a large part of the bowel is often performed for inflammatory or vasculatory disturbances. Obstructions are usually the cause of intestinal resection in the newborn.

Diarrhoea is the common postoperative complication in patients subjected to resection of the small intestine, but this can usually be controlled through parenteral fluid and electrolyte therapy and dietary measurements. Patients subjected to massive resection of the small intestine will usually show symptoms of deficiency in absorption of food stuffs.

Intestinal malabsorption following re section of the small intestine is similar to the malabsorption syndrome Poor fat absorption is one of the main problems following massive resection of the small intestine Fat absorption takes place mainly in the ileum according to most authors Nutritional studies performed by Kalser *et al.* [5] and Cornell *et al* [ ] on patients following massive resection of the small intestine have shown that the ileum is more concerned with the absorption of fat than the jejunum. Kalser *et al* compared two groups of patients subjected to massive resection of the small intestine and demonstrated that in those patients with a segment of terminal ileum and the ileocoecal valve intact, the faecal fat was only one-third of those in whom these were resected Steatorrhoea was not a large problem in our patient and was managed through restriction of fat intake

The major absorption of amino acids takes place in the small intestine but the digestion of proteins and absorption of amino acids in patients following massive resection of the small intestine is disturbed to a lesser degree than the fat absorption. Carbohydrate absorption takes place from the stomach small and large intestine particularly the proximal part of the intestine Deficient carbohydrate absorption is usually no problem following massive resection of the small intestine, but the digestion of polysaccharides may be impaired t some extent

The absorption of fat soluble vitamins is dependent on the fat absorption and a deficiency in absorption of vitamins A D E and K may be the result of steatorrhoea. Decreased plasma prothrombin was found in our case

There have been som discussion as to the site of absorption of vit min B_{12}. The studies by Kalser *et al* [5] nd Cornell *et al* [2] clearly demonstrate that the terminal ileum and ileocoecal valve are of major importance for the absor t on of B_{12} Kalser *et al* showed that 5 of 14 patients developed signs of severe megaloblastic anaemia following massive resection of the small intestine including ileocoecal valve No case of megaloblastic anaemia occurred in a similar group of patients with intact terminal ileum and ileocoecal valve Clarck & Booth [1] found a mild degree of megaloblastic anaemia in their infant t the age of 13 months. Anastomosis between jejunum and mid colon had been performed on the *0th day of life Wilkinson *et al* [9] also demonstrated vitamin B_{12} deficiency in the infant with only jejunum remaining of the small intestine while no signs of megaloblastic anaemia have been demonstrated in their other surviving infant and in Pilling & Cresson [6] two infant as well as the one reported here The terminal ileum and ileocoecal valve were intact in all four infants. There seemed to be normal B_{12} bsorption in our infant at th age of 1 year and 8 months.

Iron is bsorbed mainly in the duodenum and iron-deficiency anaemia has not been seen in the four infants following massive resection of the small intestine Massive resection of the small intestine may result in large loss of fluid and electrolytes, in particular potassium, and calcium bsorption may be impaired. Intact terminal ileum with ileocoecal valve seems to be of major importance for electrolyte and fluid bsorption in such cases. Kalser *et al* reported intract ble fluid and electrolyte

loss in an adult with 2½ feet of jejunum and jejunosigmoidostomy

It has been maintained that a remarkable growth of the small intestine may be seen following resection. The growth of the small intestine in the present case was not definitely more than corresponding to the growth of the child

Summary

Follow up on a newborn female infant *after resection of all but 30 cm of the small* intestine is reported A segment of terminal and the ileocoecal valve was intact. She showed a relatively mild degree of malabsorption syndrome during her first two years of life with temporary slightly retarded growth There were no signs of deficient iron or B_{12} absorption but her plasma prothrombin was decreased during her first year of life Her diarrhoea periods could be prevented by means of a diet low in fat and roughage. Her growth and development were completely normal at the *age of 2 $\frac{8}{12}$ years*

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CASE REPORT

Chondro-Ectodermal Dysplasia (Ellis–van Creveld's Syndrome)

Two Certain and Two Probable Cases in the Same Family

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In 1940 R. W. B. Ellis and S. van Creveld described a new syndrome of multiple deformities with dysplasia of tissues from ectoderm and mesoderm [6]. The syndrome is characterized by (1) ectodermal dysplasia, (2) polydactyly, (3) chondrodysplasia and (4) congenital morbus cordis. The syndrome is now called Ellis–van Creveld's syndrome; at least three of the four criteria are required for the diagnosis. A total of 30 cases have been described [1, 4, 7, 8]. In 1960 Dayer [4] made an exhaustive study of the syndrome with a list of all the cases previously published. No case from Scandinavia appeared among these. Here will be briefly described a brother and sister who suffered from chondro-ectodermal dysplasia. In a later pregnancy abortion was performed on the mother. The twin foetuses showed the same skeletal changes as the living brother and sister.

Case Reports

K. N. (♂), born in 1957, and his sister A. N. (♀), born in 1956 (Case Nos. 1356 and 1357/1962 respectively), were transferred to the Department of Paediatrics from the Department of Dermatology, University Hospital, Uppsala, for investigation because of a poor growth of hair and stunted development. There was no evidence of inherited arrested growth or body deformity. Both parents came from Finland, were healthy and of normal size. No consanguinity existed between the parents. When the boy was born, the mother was 22 years of age and the father 25. There were no other siblings. The two pregnancies and deliveries were without complications. At birth short clumsy arms and legs were noted in both infants.

Length at birth: ♂ 44 cm, ♀ 41 cm

Weight at birth: ♂ 2920 g, ♀ 2930 g

The development of growth was retarded (see Fig. 1). The psychomotor development was completely normal. Previously the two children had been treated in hospital for coryzal infections and a diagnosis of chondrodystrophy was made. Apart from infections and pyloric stenosis (♂) they had usually been healthy.

Physical examination at the ages of $5\frac{1}{12}$ (♂) and 4 (♀) years respectively: Length 82 cm (♂) and 74 cm (♀) (normal for the ages 110 and 102 cm). The hair was brittle and thin, "like cotton-grass" (Fig.). The nails were dysplastic, brittle, furrowed and

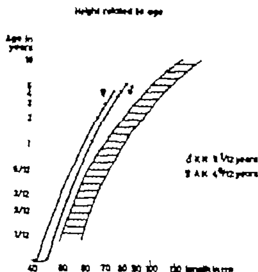


Fig. 1 Development in length relative to age for the brother and sister with the Ellis-van Creveld's syndrome. The lined area indicates the range of variation for normal Swedish children (Karlberg, P and Persson, A. *Acta Paediat (Stockh)*, *Suppl 117* 128, 1959; Broman B Dahlberg, G and Lichtenstein, A. *Acta Paediat (Stockh)*, *30* 1 1942).

short. The skin was dry The activity of the sweat-glands was reduced over the whole trunk in both children; iontophoresis in increasing doses showed no sweating in the boy except for his head. The function of the lacrimal glands was obviously reduced in the right eye of the boy (Fig) The inferior maxillae were somewhat small Teeth and dental germs were normal. The extremities were short curved, with a broadening of the metaphyses (Fig. 2) The hands were short and broad; no polydactyly

In both children the radiological changes of the skeleton (Fig. 3) were of the same localisation and character Changes were not observed in the skull, spine or pelvis, but were restricted to the bones of the extremities, more pronounced in the legs than the arms.

In all respect the long bones were short and relatively broad. The length estimated as a percentage of normal values (9) is as follows.

Humerus ♂ 65 %, ♀ [illegible] %; Radius ♂ 66 % ♀ 60 %; Ulna ♂ 67 % ♀ 66 %

Femur ♂ 64 %, ♀ 64 %; Tibia ♂ 66 % ♀ 57 %; Fibula ♂ 56 % ♀ 59 %

The metacarpus, metatarsus and phalanges of both hands and feet were short and relatively broad. Apart from these abnormalities there was nothing of note in the upper limbs.

In the lower limbs there was a lateral convexity of both femora and a coxa valga of the hip. The metaphyses showed an obvious widening especially in the knee and ankle joints irregular towards the epiphyses while within the metaphyses there were irregular rarefactions. The epiphyses showed no changes

In both patients the development of the bone nuclei calculated according to Elgenmark (5) was somewhat retarded The number of bone nuclei in the boy was 56 with a normal scatter of 58–65, and in the girl 50 with a normal scatter 1 55–67

In neither of those cases was there polydactyly polymetacarpalism, synmetacarpalism or syncarpalism, nor the lesion of the proximal part of the tibia described by Caffey (3) as pathognomonic. In all previous cases a broadening of the metaphysis had been observed with a relatively long lateral slope and a short medial slope In addition, the medial slope was covered with a thin hypoplastic epiphyseal nucleus. In our cases the proximal border of the metaphysis as straight and at right angles to the length of the tibia and the epiphyseal nucleus was normally developed.

Radiological examination of the heart in the two children showed moderate enlargement, but without the typical changes of congenital heart disease. The sizes of the hearts were 430 (♂) and 455 ml/m body surface (♀) respectively (Normal values for their ages are 310 and 305 ml/m respectively) The auscultatory findings and the electrocardiogram were quite normal.

Renal function tests and intravenous pyelography were normal.

The following tests were normal in all

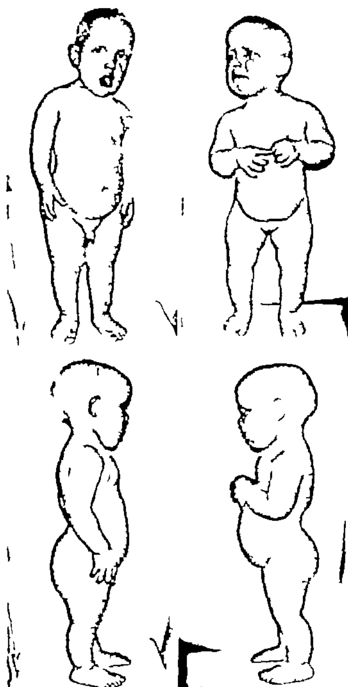


Fig. — A brother and sister with the Ellis-van Creveld syndrome. The extremities are short and clumsy, especially distally; in the usual chondro-dystrophy the shortening is most pronounced proximally.

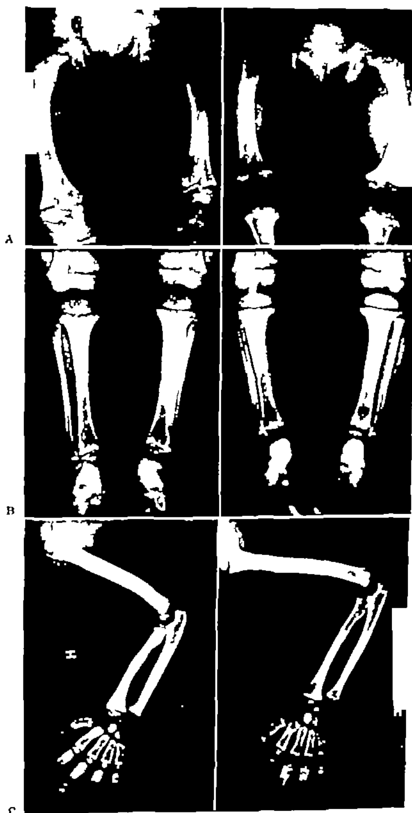


Fig. 2. (A) The femora are short and curvy with a lateral convexity; bilateral coxa vara. The knee metaphyses are broad with irregular rarefactions and with irregular borders toward the normal epiphyses. (B) Tibia and fibula are remarkably short. Changes in the metaphyses distally as in Fig. 3A. Short and somewhat broadened metatarsals. (C) The bones of the upper arm and forearm are short; phalanges and metacarpals short and relatively broad. Boy left, girl right.



Fig 4 (A) Twins in the 8th month of pregnancy. Femora are remarkably short and curved with lateral convexity. Skeletal X-ray otherwise normal. (B) and (C) The bone-cartilage interface in the femur is diffusely irregular in structure. The osseous lamellae are broad, short irregular. The cartilage cells are not normally grouped in palisades.

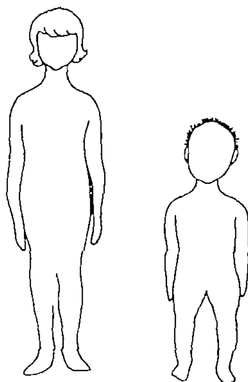


Fig 5 A girl with the Ellis–van Creveld' syndrome compared with a normally sized girl of the same age.

respects: Wassermann test, PBI, TIT cholesterol, fasting blood sugar calcium, phosphorus and phosphatase in serum serum lectrolytes electrolytes in perspiration, paper chromatography of amino acids and mucopolysaccharides in the urine. There was neither protein nor reducing substance in the urine A slight anaemia and a slightly increased sedimentation rate, probably resulting from a postinfectious condition, were found. The bone marrow showed no abnormality Motor age examination, child psychiatric and child psychological examinations with performance test showed normal levels of development and talent relative to age Classification of the sex chromatin was normal. The chromosome patterns were complet ly normal.

On reading through the hospital records it appears that the mother was granted a legal abortion in 1960· the foetuses, 5-month-old male twins, showed pathological lesions of the skeleton, which were interpreted as chondro-dystrophic, but there were no other malformations. In both foetuses, radiological examination (Fig 4A) showed obviously short femora which were rather curved with a lateral convexity but nothing else of interest. The histopathological examination (Figs. 4B and C) showed that the border between bone and cartilage in the femur was clearly irregular· the bony lamellae were short, broad and irregular· the cartilage cells were not normally grouped in palisades and the capillaries grew irrgularly into the cartilage tissue (Grøntoft)

Discussion

Apart from the combination of polydactyly ectodermal and cardiac deformities Ellis–van Creveld's syndrome implies a chondro-dystrophia different from the common type Among the 30 cases, 18 boys and 21 girls with the Ellis–van Creveld's syndrome which have been described earlier [1 4 7 8] there were 5 pairs of brothers and sisters In 11 cases consanguinity between the parents could be demonstrated Consequently the syndrome appears to be dependent on a nonsex linked recessive factor

On the other hand the usual chondrodystrophia appears in most cases by mutation and is inherited as a single dominant trait [11 12] In a Swedish material [2] the frequency of the so-called usual chondro-dystrophia is 0 0 per 1000 births. In large groups of chondro-dystrophia in Denmark [11] and Ireland [12] there is no case with polydactyly among the living children.

The impaired growth in the Ellis–van Creveld s syndrome and in the usual chondro-dystrophia is mainly due to the shortening of the extremities (Fig 5) In the usual chondro-dystrophia this shorten ing is most pronounced close to the shoul der and pelvic girdles; in the Ellis–van Creveld s syndrome on the other hand, there is a progressive distal shortening of the bones of the extremities [3] The con verse is found in Marfan s syndrome arachnodactyly with a progressive distal lengthening of the extremities.

Mentally the patients with Ellis–van Creveld s syndrome are often normal like those with the usual chondro-dystrophia and like the brother and sister described in this paper

Cardiopathy with idiopathic enlarge ment was found in the present cases In the cases with Ellis–van Creveld s syn drome previously described the cardio-pathy was a less constant component than the polydactyly and it often ppeared as atrial or ventricular septal defect The prognosis for survival depends on the nature and degree of the cardi c anomaly

Histopathological examination of one case with Ellis–van Creveld s syndrome by Uehlinger [13] showed a ventricular septal defect disturbance f cartilaginous growth, disturbance of ossification and nephrocalcinosis No pathological lesions were found in other organs Most probably the phenocritical phase occurs between the 3rd–6th weeks f pregnancy with a foetal length of 7–8 mm [13]

However the cause of this syndrom is not clear despite radiological, metaboli and chromosomal in tigations of the present cases

As the disease is recessi e both parents must be heterozygotes The probab lity that all the children in 3 pregn ancies will prove to have the disease is 1 14 if re sulting twins are monozygotes Otherwise the risk that all four children would be affected is 1 256 Four cases at three con secutive pregnancies do not contradict a recessive heredity the risk of hetero zygote parents having children with the disease is always 25 % at every new preg nancy

Summary

A brother and sister with ectodermal dysplasia cardiopathy and chondrodys-plasia are described, twin foetuses in the same family also howed chondrodyspla sia A total of 41 cases have now been described with the Ellis–van Creveld syn drome Among these there were 6 pairs of brothers and sisters Characteristics of the Ellis–van Creveld s syndrome and it dif ferences from the usual chondro-dystrophia are discussed.

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REVIEW ARTICLE

The Future of Measles Vaccination[1]

by ARVID WALLGREN

Prior to this seminar two international expert groups on measles vaccination have been convened, the first one by United States National Institutes of Health in 1961 [7], and the other by WHO in Geneva in 1963 [1.]. At each of these meetings, some views were given on the future of the vaccination. To judge from the reports of the WHO conference and at this seminar many problems regarding measles vaccination discussed at the first meeting, have already been tackled. Many problems remain, however unsolved. I am afraid that my attempt to evaluate the further development will be a kind of wishful thinking, expressing my hope how the development should be

It is not possible solely to confine a discussion about the future of vaccination against measles to the vaccination itself The future development will depend also upon other factors for instance eventual modifications of the disease, the further development of the vaccine and the method of vaccination and on the host the human being, and his degree of susceptibility and the risk h runs from the disease In the following I will try to give a few comments on these points.

[1] Paper read t he International Children Center seminar on Vaccination against Measles under the chairmanship of John F Enders, Paris, June 17 1964

Future changes of natural measles

Measles is a typical infectious disease with general susceptibility marked infectivity high pathogenicity and life-long immunity We do not know if those who acquired symptom free measles formerly were less numerous than the few who now escape clinical disease. So far there has been no changes of the infectivity of measles and we cannot therefore expect any change in future of the infectivity In former days, measles was a much more serious disease than to-day in Europe Can this be due to a change in the potency of the virus is it nowadays a more benign mutant f the old virus that is active? Probably not The more benign type of measles in our days is not due to a change of the virus itself; when imported to other parts of the world the disease runs a course corresponding with that in European countries centuries ago. We have therefore no reason to envisage a future change of the irus of measles.

The future f the vaccine

The report given at thi seminar and the ensuing discussions have hown that there is no general agreement about which kind of vaccine should be regarded s the vaccine of choice As a matter of fact, this information proves that so far nobod- t-

quite satisfied with the vaccine he has studied. The ideal vaccine should not necessitate three injections or the use of gamma globulin. It should give sufficiently high and persistent immunity be safe and free from side effects and from contamination with virus of malignancy and enjoy satisfactory public acceptance. No doubt a vaccine of this standard will be produced within the near future to judge from the intense interest virologists and immunologists all over the world show in measles vaccination.

As far as I can understand, the present inactivated vaccines alone are out of consideration, because the immunological response is slighter and the duration of immunity much shorter than with attenuated vaccines It seems improbable that the future development of an inactivated vaccine will be a more satisfactory immunizing agent than hitherto More success seems to be offered by the further development of the attenuated vaccine Before general acceptance of future vaccines, it is necessary to try them under different climatic conditions and during different seasons and to get more precise factual information about the contra indications now presented on theoretical grounds and how to tackle this problem.

Because there is serological similarity between measles virus and the virus of distemper [..] more attempts will probably be made to test the possibility of producing a measles preventive distemper virus vaccine We know that distemper does not occur as disease in man. If the production of an effective and safe vaccine would be possible, then a very important problem would be solved

The future of the method of vaccination

The present methods of measles vaccination are rather complicated to be used in a mass campaign. either three injections of inactivated measles vaccine with one month interval or one injection of attenuated virus vaccine with concomitant injection of gamma-globulin with another syringe. The further development will certainly remove the need of injection of gamma-globulin with the expected new types of attenuated vaccines, and the use of three injections of inactivated vaccine will stop because of too short immunity

Our children are subject to a great number of injections of various kinds of vaccines at the Child Welfare Centers and the more the number of injections can be reduced the better Studies about the most practical and successful type of vaccine combinations will certainly be undertaken and solve the problem of the best method of measles vaccination without an additional number of injections. In socio-economically developed countries Child Welfare Centers often use so called triple vaccinations against pertussis, diphtheria and tetanus. In order to get an early increased resistance against pertussis, the most dreaded of the three diseases, the first injection is made at about 3 months of age and the third and last injection at about 6 months. A combination of measles vaccination with this type of triple vaccination is not possible because the mother's antibodies at these early ages neutralize the antigen of measles vaccine. Further studies will be made of the possibility of introducing measles vaccine in a successful pertussis-diphtheria-tetanus type of vaccination programme

The role of the host for the future development of measles vaccination

Measles is a global problem and the future of vaccination must be evaluated with this view in mind. Even if the disease occurs and is of interest as a health problem all over the world, its significance is very different in different countries. What may be a true prediction of the future development of measles vaccination in one country will therefore not fit another country

If we first consider measles in *socio-economically highly developed countries* marked changes have occurred with regard to the significance of the disease Some centuries ago, measles was regarded as a very serious illness, causing death of thousands of children. Since then measles has become more and more benign and is at present generally regarded as a rather harmless disease in otherwise healthy children. It has turned out to be a transient evil, which parents and physicians generally accept with equanimity

Most threatened is the age-group 9 month to 3 years nd children with cute and chronic resistance-depressing disorders. The mortality rat is only bout 1 100 000 [1]. As has lready been pointed out this low mortality is not due to a less virulent agent but to other factors prophylactic injection of gamma-globulin, antibiotic treatment of respiratory complications and, bove all, increased unspecific resistance by a high standard of living. So far ccination gainst measles has not as a rule been used in these countries, except f experimental studies The question now arises: will there be ind ca tions for the introduction of a general measles vaccination of small children in these countries? In view of the insignificant risk of the formerly dreaded serious respiratory complications the reply would be: No

The risk of encephalitis puts, however the problem of vaccination in another light Formerly one heard rather little about measles encephalitis. It was the respiratory and otitic complications that dominated. When these have been effectively controlled, encephalitis has come more to the front. Its incidence is difficult to evaluate because in most countries measles is not a notifiable disease Electoencephalographic studies have shown a pathological pattern of the tracings in 50 % of all children with measles [4] and studies of the cerebro-spinal fluid have revealed pleocytosis in 10 % of ordinary cases of measles [7]. In my opinion the minute neurological lesions that produce these pathological changes without any clinical signs of encephalo-meningitis should not play a role in evaluating the need of vaccination against measles.

Clinical cases of measles encephalitis occur at about 1 1000 of hospitalized children with measles. It is well known that the fatality rate of measles encephalitis is high and that many of those who survive may suffe from protracted sequelae For the individual child the risk is slight and w ll not create any anxiety in the parents. The total number of cases of encephalitis in our country would perhaps be bout 10 annually and it would, without doubt be very desirable to prevent these Apparently they cannot be prevented by prophylactic use of gamma-globulin. The only preven t ve measure available is effective vaccination. This seems to be the only indication

for introducing measles vaccination in a socio-economically highly developed country. Measles vaccination will however probably not become a general prophylactic measure in such a country. The future vaccine will be used as combined vaccination at Child Welfare Centers, the extent of the vaccination, as in other kinds of voluntary vaccinations in children, depending upon the interest of the parents and the attending physicians. In selected groups of children, children in hospitals and other institutions, measles vaccination will be used immediately after the introduction of the first case of measles in such a group.

I will now turn to the opposite extreme of a population, which generally is called "*virgin soil population*", which by its isolation from other people has been free from measles for many years, perhaps generations. In such a population the danger of measles is very great due to several associated conditions: low general resistance of the population by frequent intermarriage and unsatisfactory general state of health, more malignant character of measles when the disease is introduced and suddenly attacks the whole population causing high mortality, especially among small children and old people.

There are several such "virgin soil" outbreaks of measles reported. The most quoted is perhaps that which occurred in the Faroe Islands and studied by Panum in 1846 [14]. Even in our days such measles epidemics have been reported from several countries, for instance Greenland [1], Canada and Polynesia [7].

At present and during the immediate future there will be a great demand for measles vaccination in such a "virgin soil" population. This vaccination should be performed already before there is any immediate threat of infection. If vaccination is not performed until the first case of measles has manifested itself, vaccination will be too late, because the infection has had time to spread to the whole susceptible population, before the first case has come to the knowledge of the public health authorities due to the remote and isolated localisation of this group of population. Public health authorities will in future pay special attention to the eventual existence of such "virgin soil" populations and to the need of prophylactic vaccination against measles. In every known "virgin soil" population the responsible health authorities should introduce measles vaccination of the whole population and later, with not too long intervals, of all children born since the last vaccination was made. This is a question of administration and of adequacy of health services of the country and would, because of the restricted number of people, not be too difficult problem to tackle.

Perhaps in a generation or two further development of communications will produce a disappearance of "virgin soil" populations and change them into the category of population, of which I now will deal with.

Most populations of the world have a borderline position between the highly developed and the "virgin soil" countries or areas. All of them belong to the so-called *developing countries* in Africa, Latin America and Asia. Measles is endemic or returns in short intervals and has a more or less high mortality rate. Some of these countries correspond to conditions prevailing in our country some centuries ago.

The degree of risk of measles people of these countries run varies depending upon their degree of socio-economic development and adequacy of their health administration and services.

Unsatisfactory state of health difficulty to get medical service great frequency of resistance-lowering illness such as tuberculosis, malaria and malnutrition, contribute to produce a high mortality rate Reports are available of case fatality rates of 5–10 % or more [7]

In these populations general measles vaccination is desirable but the administration of vaccination is very difficult due to the great ever increasing number of people lack of sufficient health personnel and to local geographical conditions The health authorities have already too much to do with their other major health problems and could not afford to take over a new responsibility They cannot in d vance evaluate if their contribution will correspond to the results achieved. A general measles vaccination is therefore out of question.

Two categories of the population are often subject to satisfactory health supervision even in developing countries labourers in industries and mines, nd children in hospitals and other institutions If it should be found that labourers recruited from remote places often fell ill with measles, vaccination should be performed by the officer of health of the industry at the start of employment It should be remembered that t is not only the question f mortality of measles which probably will not be significant but lo of the lo of working days due to the illness itself

Children in camps nurseries hospital and other institutions [illegible] be vaccinated against measles as well [illegible] other current infectious diseases [illegible] regard there is no difference betw [illegible] de-veloped and developing count[illegible] the age of elementary school at [illegible] children will as a rule already [illegible] measles and are therefore not in need [illegible] vaccination.

The problem is more difficult when it question of those parts of the population that do not live with permanent medical health supervision In these people it must depend upon the level of measles mortality whether anything should be done or not Surveys should be made to determine the degree of morbidity and mortality and the general epidemiology of measles in the population. If vaccination is to be introduced it must be a priority drawn up, with regard to the group or groups of the population, which in the first place should be vaccinated. It is, however better to proceed slowly instead of urgent measures One has to consider that neither the economic nor the personnel resources at present permit an optimisti view regarding general measles vaccination in future

Not until the socio-economic develop ment of the country has attained a degree that permits public health authorities t ffor general vaccination of the whole population can this be done At th [illegible] tainly very remote time the [illegible] of susceptible children is [illegible] proved, that measles is [illegible] major health problem it [illegible] measles vaccination could then [illegible] formed [illegible] was discussed [illegible] loped countries

Eradication of measles

The utopia of the future would be the eradication of measles. Lately there has been much talk about the eradication of malaria and tuberculosis but the aim will probably not be attained in our lifetime if ever So far it has not been possible by active measures to eradicate a single in fectious disease Theoretically however measles could be eradicated by general mass vaccination of all people all over the world. Practically this will never happen. Vaccination in the long run will not be accepted by people, who are not interested in this procedure because they have no experience of the danger of the disease The persistence of measles vaccination is therefore from psychological reasons not possible. Sooner or later people will lose interest in the procedure, oppose it, and after a certain lapse of time a gradually increasing number of the population will be unvaccinated and thus susceptible to infection. There is reason to believe that measles virus will not for ever be eradicated by general vaccination even if mass vaccination will prevent all clinical cases of the disease in human beings There may be reservoirs of virus in animals, for in stance in monkeys, and the virus may spread from these to man, if mass vaccination has stopped.

It seems to be more reasonable to try to eradicate serious types of measles by the means at our disposal improving the natural resistance of people by adequate nutrition, international support of the socio-economical development of the coun tries, promotion of public health services by education and training of health per sonnel, vaccination of susceptible institutionalized children and children suffering from any kind of disorder threatening to decrease the resistance adequate use of antibiotics against respiratory complications, etc.

Conclusions

Current scientific studies performed all over the world will probably eventually lead to the production of a vaccine against measles, that will be safe acceptable, and effective An effective and safe measles vaccine is to be regarded as a great benefit to mankind and will certainly save many thousands of children s lives.

A mass vaccination against measles will be unlikely Public Health authorities can hardly support financially or be willing to undertake or to push the introduction of this immunization in such public drives, as we have seen in vaccination against poliomyelitis. The propaganda of this vacci nation was successful Polio attacks both children and adults, and is a far greater threat against the lives and health of people than measles. It was therefore far more easy to persuade the populations to give their consent to mass-vaccination. In socio-economically developed countries measles is a rather benign disease and it is therefore not possible to persuade pa rents of the necessity of the vaccination. Measles vaccination will be a matter of decision of the individual physician attend ing child welfare centers and institutions for children. In developing countries mea sles is a more serious disease but mass vaccination of the whole population will not be possible even with international financial support due to lack of sufficient health personnel to the size of the popu

lation and to local geographical conditions. However, in selected groups of the population all over the world the vaccination against measles will play an important role in future.

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PROCEEDINGS OF PEDIATRIC SOCIETY

Pediatric Society of South Sweden

Meeting, May 10 1964

P Selander and G Theander Cholecystography in Children with Recurrent Abdominal Pains

The gallbladder in 178 children was roentgenographically examined with Bilijodon®-Natrium (sodium iopanoat) for variation in shape so-called poikilomorphosis. Such variation in adults has been found to be correlated with biliary colic Of the children studied, 8 had a history of typical biliary colic, 89 of recurrent abdominal pains (r.a.p) and 43 of abdominal colic other than r.a.p In the remaining series of 38 children no history of abdominal pain had been obtained. The incidences of poikilomorphosis recorded in the respective groups were 60, 58, 42, and 20 %. It seems probable that r.a.p. in children is not infrequently due to biliary dysfunction

H Ekel nd Asthmatoid Bronchitis in Children from 0–? Years f Age

In order t obtain an idea of the frequency and prognosis of bronchitis with asthmatoid symptoms, all the cases in children of the age group 0–2 years who have been treated between 1949–1963 at the Children s Hospital in Malmö under the diagnosis of asthmatoid bronchitis have been compiled. The criterion for the diagnosis has been infection of the upper respiratory tract which within a couple of days has been accompanied by gradually increasing expiratory dyspnea (wheezing) coughing and often fever The total number of cases was 980 of which 637 were boys and 343 girls. About three fourths of the children became ill before reaching one year of age. The most common age was 5–6 months. During the ten year period 1949–1958, 502 patients were treated and during the five-year period 1959–1963, 478 patients. A comparison with, firstly the total number of l ve births in Malmö and, secondly the hospital admittance figures for the same fifteen year period indicates that there was an absolute increase in the frequency of the disease. About three fourths of the patients had had asthmatoid bronchitis only once. Half of these were uncomplicated cases (no otitis, bronchopneumonia, etc.) and had been admitted only on this occasion. The other half had complications or had received treatment at other times for infection of the upper respiratory tract otitis or bronchopneumonia. Of the remaining one-fourth, about two-thirds had recurrences up to four times within two years after the initial occurrence of the disease, while one-third had more than four recurrences. With the help of the hospital records and questionnaires the health of the children treated during the period 1949–1958 was judged at least five years after the onset of the illness. Data could be obtained from 221 of the 502 patients. Of 147 who had had one attack, 15 had developed genuine asthma. Of 74 who had had several ttacks, 38 had developed asthma. The risk of developing asthma after one attack was therefore 10 but afte repeated attacks, 50 %. Familial allergy occurred in 20 f the 132 children who were healthy after the five-year period, in 40 % of the 15 children who had had one attack and in 60 of the 38 children who had had several ttacks and who after five years had developed genuine asthma. Of these 38 children, 90 % had become ill after seven months of age and 53 % after one year of age. Of the 38 children who had had

repeated relapses but after five years were healthy 70 % had become ill after seven months of age and 45 % after one year of age. Of 122 children who had had only one attack and after five years were healthy 55 % had become ill after seven months of age and only 15 % after one year of age The differences are statistically significant and indicate that the prognosis was poorer the older the child was at the time of the initial attack of asthmatoid bronchitis.

) Bergfors. Partial Addison's Disease

A nine-year-old girl who repeatedly had been hospitalized for vomiting and diarrhea was shown to have a marked eosinophilia. Repeated Thorn tests were pathological, scres of 17-OH-CS and 17 KS in the urine showed decreased values, and aldosterone-bearing fractions were greatly reduced The ratio Na/K was low in the serum and high in the urine She was not hyperpigmented the thyroidal and parathyroidal functions were normal and there was n candidiasis. During the ACTH infusion test she vomited heavily and there was a rapid deterioration of her general condition. Cortisol in the serum diminished rapidly meanwhile There was no increase of 17-OH-CS and 17 KS in the urine. For approximately 18 months she has been given Cortisol 20 mg/24 h and Florinef 0 1 mg/24 h. The electrolyt lues became rapidly normalized; the general condition is satisfactory as well as the rat of growth.

K. O N lsen Monarthritis

Of 12 boys and 7 girls with monarthritis the youngest was 2½ years and the eldest 14 In 17 cases the knee joint was ffected In 12 cases different infections had preceded the arthritis, and in one case the disease developed simultaneously with erythema nodosum. Fever was present in 8 cases but was quickly normalized In one-fourth of the cases hemolytic streptococci were present in the throat The sediment tion rat was elevated in 11 cases usually t more than 30 mm/h. The antistreptolysin titer was positive in 6 cases of 18 the test phylolysin in 13 f 16, C-react e protein in 6 of 16 Gamma globulin was increased 4 and th alpha fraction in 10 of 13 examined cases. All cases were negati e for gglut nat on of sensitized sheep-blood cells. Roentgen examination revealed changes in the joint n only one case Examination of 16 p tien after an verage of 4.3 years showed h t 13 were entirely asymptomatic

P Selander and A Toreld Encopresis

Of 180 cases of encopresi t the Children's Clinic in Malmö 72 % were boys Forty per cent had primary encopresis 3 began fecal soiling after 11 years of age. Treatment w sought earlier for girls than for boys. Encopresis was probably most common in the lowest social class (social group III). Obst pation, enuresis and nervous symptoms were present in 37 43 and 60 % of the cases, respectively A follow-u p revealed that 61 % had been entirely cured after only internal pediatric treatment (laxatives, enema). A comparison of the cured and non-cured cases showed no significant difference as t sex, ge primary versus secondary encopresis, obstipation, concomitant nervous symptoms or intellectual retardation. Few of the children had psychoneurosis, but among the prolonged and most refractory cases there was a significant preponderance of bnormal personalities.

L. Eriksen Spontaneously Healed Meconium Peritonitis

The boy is the fourth child of healthy parents. The pregnancy and birth were normal. The liquor amnii was thick and yellowish green. It was bserved at birth that the child had marked contractions of the epigastrium during inspiration. Roentgen examination revealed extensive calcifications in the bdomen typical of meconium peritonitis. The same changes were found in the scrotum. Calcium was found in the right cupola of the diaphragm; here the diaphragm was considerably shrivelled and drawn up. Roentgenography revealed no

passage of the contrast medium out into the abdominal cavity After a couple of weeks the respiration began to be normal. Roentgen examination at 3½ months of age showed unchanged calcifications; the diaphragm was less drawn up At 17 months of age the boy was clinically healthy A roentgen examination showed considerable regression of the calcifications. Of about 200 reported cases of meconium peritonitis 3 have healed spontaneously

H Eklund and G Kreek "Collodion Baby"

"Collodion baby" (lamellar ichthyosis in new born) is a rare congenital anomaly It is often familial The child is born with a collodion like membrane which covers it entirely or partly It can be so extensive that breathing and feeding are difficult. Other malformations occur Not few of such babies die shortly after birth; those surviving develop ichthyosis. The child in question is the first born of healthy parents with no known heredity for such a disease. At birth the boy's skin was blood-red, shiny tightly drawn especially over the joints, the movement of which was impeded. Edema developed, especially in the hands and feet. After only a few days the skin dried and scaled off A histological examination showed hyperkeratosis. The treatment was local, soft ointments being used. The boy survived but has now one year later mild form of ichthyosis.

Per Selander Malmö

BOOK REVIEW

G Müller Der plötzliche Kindstod. Pathologische Anatomie und Dynamik.

Thieme Verlag, Stuttgart 1963 148 pp. and 56 illustrations Price DM 29 70

Sudden death in infancy and childhood is a major problem in pediatrics and also in pediatric pathology as its impact is very poorly compensated by the meagre clinical and morphologic finding in these cases. Dr Müller monograph concerns the autopsy findings in 89 children who, from apparent health, suddenly died or were found dead The author considers the cause of death established in no less than 85 cases. The most common cause was upper respiratory infection (60 7 %) other infections together constituting 24 7 Only 5 instances of cardiovascular malformations were found. In most cases of respiratory infection the findings were very slight in the lungs but they were associated with a prominent cerebral edema which the author considers the final cause of death.

In half of the case-material necrobiotic changes in the lymphoid organs were found pointing to a fulminating toxic mechanism The author is very cautious in evaluating the microbiologic findings. Thirty four cases were studied virologically and in 7 there was a cytopathogenic effect one of these displayed ECHO virus. The main thesis is that in the majority of cases there is a toxic stress reaction, notably in the lymphoid apparatus. Although the relevance of these findings is supported by the absence of lymphoid tissue reaction in a control material (8 cases of accidental death) its relative frequency seems remarkable In a large case series of sudden death in infancy and childhood one would perhaps have expected a more scattered distribution of diseases It is noteworthy that no abnormalities of the pulmonary arteries with right heart hypertrophy no tumors or lesions of the islet of the pancreas were encountered.

With its excellent illustrations, good typography experimental findings and lucid pathologic correlation the monograph is good handbook, particularly for the pediatric pathologist

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